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(54) Title: PRODUCTION OF VIRAL RESISTANT PLANTS VIA INTRODUCTION OF UNTRANSLATABLE PLUS SENSE VIRAL RNA					
(57) Abstract					
Plants, such as tobacco, are made resistant to potyvirus infection by transformation with vectors which include a gene, derived from a potyvirus, mutated to encode an untranslatable plus sense RNA molecule. Mutagenized potyvirus genes and plant transformation vectors containing these genes are also disclosed.					

"Production of viral resistant plants via introduction of untranslatable plus sense viral RNA"

FIELD OF THE INVENTION

5        This invention is directed to the production of plants with a reduced susceptibility to virus infection.

BACKGROUND OF THE INVENTION

Plant viruses are responsible for major losses in worldwide crop production. Much effort is directed 10 towards the development of new plant varieties which exhibit increased resistance to viral infection. Until recently such efforts were primarily based on the traditional plant breeding approach, however this approach is often limited by a lack of sources of 15 resistance within the crop species. The advent of modern molecular biology techniques has facilitated the development of new methods of rendering plant varieties resistant to virus attack that are not limited by a requirement for preexisting resistance genes within a 20 species.

Molecular Approaches

Many of these molecular approaches are based on the theory of pathogen derived resistance (Sanford and Johnston, 1985). This theory predicts that a "normal" 25 host (plant) - pathogen (virus) relationship can be disrupted if the host organism expresses essential pathogen derived genes. It has been proposed that host organisms expressing pathogen gene products in excess amounts, at an inappropriate developmental stage, or in 30 a dysfunctional form may disrupt the normal replicative cycle of the pathogen and result in an attenuated or aborted infection of the host.

Two approaches typify this pathogen derived resistance: coat protein mediated resistance and 35 antisense RNA expression. It has been demonstrated that transgenic plants expressing a plant virus coat protein can be resistant to infection by the homologous virus. This coat protein mediated resistance has been

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replication mechanism involves both translation of the plus sense RNA to yield viral gene products (which include a replicase and a proteinase) and also the synthesis of a minus sense RNA strand. This minus sense 5 strand then acts as a template for the synthesis of many plus sense genomes which are subsequently encapsidated in coat protein to yield infectious mature "virions," thus completing the replicative cycle of the virus.

Experiments have been reported in which 10 transgenic plants expressing the coat protein gene of a potyvirus show a reduced susceptibility to virus infection (Lawson et al. 1990; Ling et al. 1991; Stark and Beachy 1989).

#### SUMMARY OF THE INVENTION

15 The disclosed invention concerns a method of producing plants with a decreased susceptibility to virus infection. This is achieved by transforming plants with a DNA molecule which includes a gene derived in part from the genome of a plant virus. This gene is 20 specifically constructed to produce an untranslatable version of a plus sense RNA molecule required for viral replication. Thus, expression of the gene within the plant causes the production of this non-functional molecule which then inhibits viral replication within 25 the plant, rendering the plant resistant to viral infection.

In particular, invention provides an alternative and novel approach to rendering plants 30 resistant to potyvirus infection.

Plants are transformed with a gene construct engineered to express an untranslatable form of the plus sense RNA which encodes the coat protein of a potyvirus.

In the case of Tobacco Etch Virus (TEV), it is demonstrated that tobacco plants transformed with such a 35 gene construct accumulate the untranslatable plus sense RNA but do not produce detectable levels of the coat protein. It is further shown that these plants are resistant to TEV infection. It is also shown that

with the restriction enzyme *Nco*I, the DNA fragment delineated by the restriction enzyme sites at B and C was removed, and the plasmid religated to generate pTC:FL. pTC:FL contains the Tobacco Etch Virus (TEV) 5 coat protein nucleotide sequence flanked by *Bam*HI restriction sites and the TEV 5' and 3' untranslated sequences (UTS). T7 and SP6 promoters are also shown. Abbreviations used in this diagram are as follows: T7, T7 RNA polymerase promoter sequence; SP6, SP6 RNA polymerase promoter sequence; ori, origin of replication; M13 ori, bacteriophage M13 single-stranded origin of replication; amp<sup>r</sup>, β-lactamase gene. Lightly stippled areas are TEV 5' and 3' untranslated sequences; solid black area, TEV genome cDNA nucleotides 144 to 10 200; striped area, a portion of the TEV NIb gene (TEV nt 8462-8517); heavily stippled areas, cDNA of TEV CP 15 nucleotide sequence (TEV nt 8518-9309).

Fig. 3 is a schematic representation of the forms of the Tobacco Etch Virus coat protein gene inserted into tobacco in the invention. All constructs contained the enhanced CaMV 35S (Enh 35S) promoter, CaMV 35S 5' untranslated sequence (UTS) of 50 bp and the CaMV 35S 3' UTS/polyadenylation site of 110 bp. The nomenclature used to describe the transgenic plant lines is presented along with the gene products produced in those plant lines (far right column). Abbreviations are as follows: 35S, transgenic plants containing the CaMV 35S promoter and 5' and 3' UTS only; FL, transgenic plants containing the transgene coding for full-length, 20 AS and RC transgenic plants contain the transgene expressed as an antisense form of the TEV CP gene, or an untranslated sense form of the TEV CP gene, respectively. Stippled areas represent various forms of the TEV CP nucleotide sequence.

Fig. 4 is a graphic representation of the appearance of systemic symptoms in plants infected with Tobacco Etch Virus showing responses of control plants and transformed plants generated as described in the

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Minus sense RNA: That form of RNA used as a template for plus sense RNA production.

Antisense RNA: RNA complementary to plus sense RNA form.

5 R<sub>0</sub> generation: Primary transformants.

R<sub>1</sub> generation: Progeny of primary transformants.

R<sub>2</sub> generation: Second generation progeny of R<sub>0</sub> generation (i.e., progeny of R<sub>1</sub> generation).

10 A gene derived in part from a plant virus RNA molecule: At least the portion of the gene encoding the untranslatable RNA molecule is derived from a plant virus RNA molecule.

GENERAL DESCRIPTION

15 An untranslatable plus sense RNA molecule is encoded by a gene located on the DNA molecule. The gene comprises DNA derived from a plant virus RNA genome and also DNA from heterologous sources. The DNA from heterologous sources includes elements controlling the expression of the virus-derived DNA sequences. The DNA sequence of the gene is specifically altered so as to render the RNA molecule transcribed from the gene untranslatable. The presence of this untranslatable plus sense RNA within the cells of the transformed plant 25 reduces the susceptibility of the plant to viral infection.

More particularly, the portion of the gene which comprises DNA from a plant virus has been derived from a potyvirus. Plants transformed with the DNA 30 molecule containing the gene are less susceptible to infection by potyviruses. Most specifically, the DNA from the potyvirus source has been derived from the coat protein gene of Tobacco Etch Virus and transformed plants are resistant to infection by Tobacco Etch Virus. 35 Plants which can be made resistant to potyvirus infection include, but are not limited to, tobacco.

Accordingly, the present invention provides a method for genetically engineering plants by insertion,

experimental example given and will appreciate the wider potential utility of the invention.

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' nontranslated region which causes polyadenylate nucleotides to be added to the 3' end of the viral RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. Promoters which are known or are found to cause transcription of viral RNA in plant cells can be used in the present invention. Such promoters may be obtained from plants or viruses and include, but are not limited to, the CaMV 35S promoter. As described below, it is preferred that the particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of untranslatable plus sense RNA to render the plant substantially resistant to virus infection. The amount of untranslatable plus sense RNA needed to induce resistance may vary with the plant type. Accordingly, while the 35S promoter is preferred, it should be understood that this promoter may not be the optimal one for all embodiments of the present invention. Furthermore, the promoters used in the DNA constructs of the invention may be modified, if desired, to affect their control characteristics. DNA sequences have been identified which confer regulatory specificity on promoter regions. For example, the small subunit of the ribulose bis-phosphate carboxylase (ss

protein gene. Furthermore, it will be apparent that suitable genomic portions are not limited to complete gene sequences.

A disclosed embodiment of the invention utilizes a double-stranded complementary DNA (cDNA) derived from the region of the TEV genome encoding the coat protein gene. To the 5' end of this cDNA is ligated the CaMV 35S promoter and CaMV 35S RNA 5' nontranslated region. To the 3' end is ligated the CaMV 35S 3' nontranslated region. These 5' and 3' sequences are present to cause transcription of the gene in plant cells by the cellular enzyme RNA polymerase to produce an RNA molecule of sequence corresponding to the sequence of the coat protein cDNA sequence. Ordinarily, such an RNA would then be translated by ribosomes which would synthesize a protein of amino acid sequence specified by the nucleotide sequence of the RNA molecule. Particular amino acids are specified by nucleotide triplets termed codons. Codons which stipulate translation initiation and termination are also present in DNA and RNA sequences. The current invention relates to RNA molecules which are untranslatable by ribosomes. In the preferred embodiment the sequence of the TEV cDNA encoding the coat protein is mutated by a standard *in vitro* mutagenesis technique to produce a frameshift mutation early in the coat protein structural gene immediately followed by three translation termination signal codons. These mutations do not affect the ability of RNA polymerase to transcribe an RNA molecule from the cDNA but prevent translation of the transcribed RNA by ribosomes. Those skilled in the art will recognize that for the disclosed gene and for other genes, DNA sequences can be altered in other ways to cause the DNA to encode an untranslatable plus sense RNA molecule. Thus the disclosed invention is not limited to the mutations disclosed.

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the TEV coat protein gene are combined in a single cloning vector. This vector is subsequently transformed into AT cells and the resultant cells are used to transform cultured tobacco cells.

5        Vectors suitable for the AT mediated transformation of plants with the DNA of the invention are disclosed. It will be obvious to one skilled in the art that a range of suitable vectors is available, including those disclosed by Bevan (1983),

10      Herrera-Estrella (1983), Klee (1985) and EPO publication 12,516 (Schilperoort et al.). Suitable vectors are available on a commercial basis from Clontech (Palo Alto, CA) and Pharmacia LKB (Pleasant Hill, CA) and other sources.

15      Following the transformation of plant cells and regeneration of transformed plants with the DNA molecules as described, regenerated plants are tested for increased virus resistance. Plants are preferably exposed to the virus at a concentration within a range where the rate of disease development correlates linearly with virus concentration. Methods for virus inoculation are well known to those skilled in the art and are reviewed by Kado and Agrawal (1972). One such method includes abrading a leaf surface with an aqueous suspension containing an abrasive material such as carborundum and virus or dusting leaves with such an abrasive material and subsequently applying the virus onto the leaf surface. A virus suspension can be directly inoculated into leaf veins or alternatively

20      plants can be inoculated using insect vectors. The virus suspension may comprise purified virus particles, or alternatively, sap from virus infected plants may be utilized.

25      Transformed plants are then assessed for resistance to the virus. The assessment of resistance or reduced susceptibility may be manifest in different ways dependant on the particular virus type and plant type. Those skilled in the art will realize that a

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CDNA. Plasmid DNA was isolated from colonies which hybridized with the probe, and the SalI/EcoRI cDNA inserts were sized by electrophoresis in a 0.8% (w/v) agarose gel using a horizontal water-cooled gel apparatus.

The SalI/EcoRI inserts from the recombinant molecules were isolated from an agarose gel with NA45 membrane (Schleicher & Schuell, Keene, NH) according to the manufacturer's protocol. The following restriction enzymes were used either alone or in combination to digest the isolated cDNA insert: HindIII, XbaI, AluI, HaeIII, RsaI, Sau3A, and *Taq*I. Restriction enzyme digestion products were inserted into the DNA of an appropriate M13 bacteriophage (Messing 1983) selected for the presence of corresponding polylinker restriction sites, and their nucleotide sequences were determined by dideoxy chain termination.

Plasmid pTL 37/8595 (Carrington and Dougherty 1987; Carrington et al. 1987, herein incorporated by reference) contains a cDNA copy of the genomic sequence of HAT TEV corresponding to nucleotides (nt) 1-200 and nt 8462-9495 (Fig. 2). (Numbering of the TEV genome nucleotides is according to that presented in Allison et al. 1986). The nucleotide sequence and deduced amino acid sequence of the Tobacco Etch Virus genome and the numbering system utilized by Allison et al. (1986) and herein is shown in Fig. 1 and SEQ ID No. 1 in the attached sequence listing. The first and last codons of the coat protein (CP) coding region in the TEV genome are nt 8518-8520 (encoding the amino acid serine) and 9307-9309 (opal stop codon) respectively. pTL 37/8595 was subject to *in vitro* site-directed mutagenesis as described by Taylor et al. (1985a, 1985b) herein incorporated by reference. In all cases, nucleotide changes were confirmed by dideoxy-nucleotide sequencing (Sanger et al. 1977).

TEV nt 9312-9317 were first mutated (Fig. 2) to generate a BamHI restriction site (GGATCC). TEV nt

no detectable protein products, while transcripts from pTC:FL produced proteins of the expected sizes.

The various forms of the CP nucleotide sequence were then inserted as BamHI cassettes into the plant 5 expression vector pPEV (see below and Fig. 3).

The full length TEV CP open reading frame of pTC:FL was inserted in the reverse orientation to make the antisense (AS) construct pTC:AS. The nucleotide sequence of the TEV CP gene in pTC:AS is shown in SEQ ID 10 No. 4 in the attached sequence listing.

#### Transformation Vector Construction

Construction of pPEV. The vector pPEV is part of a binary vector system for *Agrobacterium tumefaciens* mediated plant cell transformation. Plasmid pPEV was 15 constructed from the plasmids pCGN 2113 (Calgene), pCIB 710 and pCIB 200 (Ciba Geigy Corp.). pCGN 2113 contains the "enhanced" Cauliflower Mosaic Virus (CaMV) 35S promoter (CaMV sequences -941 to 90/-363 to +2, relative to the transcription start site) in a pUC derived 20 plasmid backbone. pCIB 710 has been described (Rothstein et al. 1987) and pCIB 200 is a derivative of the wide host range plasmid pTJS 75 (Schmidhauser and Helinski 1985) which contains left and right *A. tumefaciens* T37 DNA borders, the plant selectable 25 NOS/NPT II chimeric gene from the plasmid Bin 6 (Bevan 1984) and part of a pUC polylinker. The small EcoRI-EcoRV DNA fragment of pCIB 710 (Rothstein et al. 1987) was ligated into EcoRI-EcoRV digested pCGN 2113. This regenerated the enhanced CaMV 35S promoter (Kay et 30 al. 1987) of pCGN 2113 and introduced the CaMV 35S 5' and 3' untranslated sequences into pCGN 2113. The CaMV 35S promoterterminator cassette of the resulting plasmid was isolated as an EcoRI-XbaI DNA fragment and ligated 35 into EcoRI-XbaI digested pCIB 200 to generate pPEV. CP nucleotide sequences from PTC:FL, pTC:RC, and pTC:AS were cloned as BamHI cassettes into BamHI digested pPEV and orientation of inserts confirmed by digestion with appropriate restriction endonucleases.

isolated from tissue and RNA precipitated with LiCl as described by Verwoerd et al. (1989). RNAs were electrophoretically separated on 1.2% agarose gels containing 6% (v/v) formaldehyde and transferred to 5 nitrocellulose. Prehybridization and hybridization conditions were as described in Sambrook et al. (1989). Strand specific riboprobes were generated from SP6 or T7 DNA dependent RNA polymerase transcription reactions of pTL 37/8595 linearized with the restriction enzymes 10 Asp718 (Boehringer Mannheim, Indianapolis, IN) or HindIII, respectively, using  $\alpha$ -labelled  $^{32}\text{P}$ -CTP ribonucleotide and suggested procedures (Promega, Madison, WI).

An RNA transcript of approximately 1,000 nt was 15 expected with all transgenic plant lines. Such a TEV CP transcript was detected in CP expressing plant lines by using a minus sense riboprobe containing the TEV CP sequence. A similar transcript was detected in AS plants by using a plus sense riboprobe containing the 20 TEV CP sequence. The transcript in the RC line, while detected with a minus sense riboprobe, may have migrated as a slightly larger (ca 1,100-1,200 nt) RNA species, possibly due to termination at an alternately selected site and/or a longer poly-A tail on the transcript. 25 Differing levels of CP transcript accumulation were observed among different transgenic plant lines. Transgenic plant lines expressing the coat protein of TEV were identified by western blot analysis using polyclonal antisera to TEV CP. Tissue samples of 30 regenerated plants were ground in 10 volumes of 2X Laemmli (Tris-glycine) runner buffer (Laemmli 1970) and clarified by centrifugation in a microcentrifuge for 10 min. at 10,000xg. Protein concentration was estimated by the dye binding procedure of Bradford (1976) using 35 BSA as a standard. Protein samples (50  $\mu\text{g}$  total protein) were separated on a 12.5% polyacrylamide gel containing SDS and subjected to the immunoblot transfer procedures described by Towbin et al. (1979). Anti-TEV

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rat (Fig. 4A). Plant lines which expressed FL TEV CP showed little or no delay in the appearance of symptoms when inoculated with infected plant sap. However, FL transgenic plants did show a slight attenuation of 5 symptoms and eventually (2-4 weeks after initial appearance of symptoms), younger leaf tissue emerged devoid of symptoms and virus as demonstrated by back inoculation experiments. Typically chlorosis and etching on older systemic leaves was limited.

10 Ten independently transformed RC lines and seven independently transformed AS lines were obtained. Progeny from three of the RC lines, including line RC #5 and from one of the AS lines, including AS #3, showed an altered response to viral infection relative to control 15 plants. All of these lines were verified to be transformed and were producing expected RNA products. A possible explanation for the variation in observed phenotype is the previously noted "position effect" whereby the expression of genes from identical DNA 20 sequences integrated at different locations within the genome show varying patterns of tissue specificity.

Ten R2 expressing plants of the FL expressing line were inoculated with infected plant sap, and 20 R1 plants of lines AS #3 and RC #5 were inoculated with 25 50 µl of a 5 µg/ml solution of purified TEV. Identical results to those obtained by purified TEV inoculation were obtained when AS #3 and RC #5 R1 plants were inoculated with TEV-infected plant sap, as described above.

30 Transgenic Burley 49 plant lines AS #3 and RC #5, expressing only TEV CP related RNA sequences, showed a delay in the appearance of symptoms and a modification of symptoms when inoculated with TEV (Fig. 4B). Since the 20 R1 plants were not screened for expression of CP 35 RNA prior to inoculation, some of the symptomatic plants represented non-expressing plants in which the gene of interest had been lost during Mendelian segregation. Modified symptoms on AS #3 plants appeared as small

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and  $5 \times 10^4$  extracted protoplasts were then subjected to western blot analysis as described above. Protoplast viability was measured by dye exclusion as described in Luciano et al. (1987). All electroporated protoplast samples had equivalent viability counts. The results indicated that protoplasts from all FL plant lines supported virus replication at levels comparable to wild type Burley 49 protoplasts. R1 transgenic plants from lines AS #3 and RC #5 were initially screened by northern analysis, and leaves from positive expressors were used in the production of protoplasts. Transfected protoplasts derived from AS #3 plants supported TEV replication, albeit at a reduced level. Protoplasts derived from RC #5 transgenic plant leaf tissue did not support TEV replication at a detectable level. These results, and those presented in the whole plant inoculation series, suggested AS and RC plants interfere with TEV replication.

Discussion of Data

The above example indicates that varying degrees of protection from TEV infection can be achieved by overexpression of coat protein and by expression of an antisense RNA. The current invention which comprises the expression of an untranslatable plus sense RNA molecule provides protection against TEV infection that is more effective than either of these two methods. Plants of line RC #5, transformed with the disclosed DNA molecule encoding an untranslatable plus sense RNA derived from the TEV coat protein gene, were asymptomatic and appear to be completely protected from virus infection. The disclosed invention therefore represents a new and effective way of generating potyvirus resistant germplasm.

Tobacco protoplasts derived from plants expressing the antisense RNA supported a reduced level of TEV replication compared to control cells derived from untransformed plants. In contrast, tobacco protoplasts derived from plants of line RC #5,

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It will also be apparent to one skilled in the art that the described invention may also be used to produce plants resistant to viruses outside of the potyvirus family in instances where these viruses also 5 produce a minus sense RNA replicative template.

BIBLIOGRAPHY

Allison et al. 1985a. Virology 147:309-316.

Allison et al. 1985b. Proc. Natl. Acad. Sci. USA 82:3969-3972.

10 Allison et al. 1986. Virology 154:9-20.

Bevan 1984. Nucl. Acids Res. 12:8711-8721.

Bradford 1976. Nucl. Acids Res. 12:8711-8721.

Carrington and Dougherty 1987. J. Virol. 61:2540-2548.

15 Carrington et al. 1987. Nucl. Acids Res. 15:10066.

Ditta et al. 1980. Proc. Natl. Acad. Sci. USA 77:7347-7351.

Domier et al. 1986. Nucl. Acids Res. 14:5417-5430.

20 Dougherty and Hiebert 1980a. Virology 101:466-474.

Dougherty and Hiebert 1980b. Virology 104:183-194.

25 Hanahan and Meselson 1980. Gene 10:63-67.

Herrera-Estrella et al. 1983. Nature (London) 303:209-213.

Hollings and Brunt 1981. Commonwealth Mycological Institute/Association of Applied Biologists Descrip. Plant Viruses, No. 245.

30 Horsch et al. 1985. Science 227:1229-1231.

Kado and Agrawal 1972. Principles and Techniques in Plant Virology.

Kay et al. 1987. Science 236:1299-1302.

35 Kozak 1984. Nature (London) 308:241-246.

Kurtz and Nicodemus 1981. Gene 13:145-152.

Laemmli 1970. Nature (London) 227:680-685.

Lain et al. 1989. virus Res. 13:157-172.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

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5 (ii) TITLE OF INVENTION: Production of Plants Showing Immunity to Viral Infection via Introduction of Genes Encoding Untranslatable Plus Sense RNA Molecules

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15 (v) COMPUTER READABLE FORM:  
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20 (B) COMPUTER: IBM PC Compatible  
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(D) SOFTWARE: WordPerfect 5.1

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40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9495  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single

- 29 -

	AGC GCA CAT ATG GCT GGA GCG AAT GGA AGC ATT TTG AAG AAG GCA GAA Ser Ala His Met Ala Gly Ala Asn Gly Ser Ile Leu Lys Lys Ala Glu 30 35 40	270
5	GAG ACC TCT CGT GCA ATC ATG CAC AAA CCA GTG ATC TTC GGA GAA GAC Glu Thr Ser Arg Ala Ile Met His Lys Pro Val Ile Phe Gly Glu Asp 45 50 55	318
10	TAC ATT ACC GAG GCA GAC TTG CCT TAC ACA CCA CTC CAT TTA GAG GTC Tyr Ile Thr Glu Ala Asp Leu Pro Tyr Thr Pro Leu His Leu Glu Val 60 65 70	366
15	GAT GCT GAA ATG GAG CGG ATG TAT TAT CTT GGT CGT CGC GCG CTC ACC Asp Ala Glu Met Glu Arg Met Tyr Tyr Leu Gly Arg Arg Ala Leu Thr 75 80 85 90	414
20	CAT GGC AAG AGA CGC AAA GTT TCT GTG AAT AAC AAG AGG AAC AGG AGA His Gly Lys Arg Arg Lys Val Ser Val Asn Asn Lys Arg Asn Arg Arg 95 100 105	462
25	AGG AAA GTG GCC AAA ACG TAC GTG GGG CGT GAT TCC ATT GTT GAG AAG Arg Lys Val Ala Lys Thr Tyr Val Gly Arg Asp Ser Ile Val Glu Lys 110 115 120	510
30	ATT GTA GTG CCC CAC ACC GAG AGA AAG GTT GAT ACC ACA GCA GCA GTG Ile Val Val Pro His Thr Glu Arg Lys Val Asp Thr Thr Ala Ala Val 125 130 135	558
35	GAA GAC ATT TGC AAT GAA GCT ACC ACT CAA CTT GTG CAT AAT AGT ATG Glu Asp Ile Cys Asn Glu Ala Thr Thr Gln Leu Val His Asn Ser Met 140 145 150	606
40	CCA AAG CGT AAG AAG CAG AAA AAC TTC TTG CCC GCC ACT TCA CTA AGT Pro Lys Arg Lys Lys Gln Lys Asn Phe Leu Pro Ala Thr Ser Leu Ser 155 160 165 170	654
45	AAC GTG TAT GCC CAA ACT TGG AGC ATA GTG CGC AAA CGC CAT ATG CAG Asn Val Tyr Ala Gln Thr Trp Ser Ile Val Arg Lys Arg His Met Gln 175 180 185	702
50	GTG GAG ATC ATT AGC AAG AAG AGC GTC CGA GCG AGG GTC AAG AGA TTT Val Glu Ile Ile Ser Lys Ser Val Arg Ala Arg Val Lys Arg Phe 190 195 200	750
55	GAG GGC TCG GTG CAA TTG TTC GCA AGT GTG CGT CAC ATG TAT GGC GAG Glu Gly Ser Val Gln Leu Phe Ala Ser Val Arg His Met Tyr Gly Glu 205 210 215	798
60	AGG AAA AGG GTG GAC TTA CGT ATT GAC AAC TGG CAG CAA GAG ACA CTT Arg Lys Arg Val Asp Leu Arg Ile Asp Asn Trp Gln Gln Glu Thr Leu 220 225 230	846
65	CTA GAC CTT GCT AAA AGA TTT AAG AAT GAG AGA GTG GAT CAA TCG AAG Leu Asp Leu Ala Lys Arg Phe Lys Asn Glu Arg Val Asp Gln Ser Lys 235 240 245 250	894
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80	GAT GGG ATG TTG GTG GAT GCT CGT GCG AAG GTA ACG TTC GCT GTT TGT Asp Gly Met Leu Val Asp Ala Arg Ala Lys Val Thr Phe Ala Val Cys 285 290 295	1038

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5	TCA ATG CGG AAT GGT AAT TAC GTG TAC CCA TGT TGT TGT GTT ACT CTT Ser Met Arg Asn Gly Asn Tyr Val Tyr Pro Cys Cys Cys Val Thr Leu 590 595 600	1950
10	GAA GAT GGT AAG GCT CAA TAT TCG GAT CTA AAG CAC CCA ACG AAG AGA Glu Asp Gly Lys Ala Gln Tyr Ser Asp Leu Lys His Pro Thr Lys Arg 605 610 615	1998
15	CAT CTG GTC ATT GGC AAC TCT GGC GAT TCA AAG TAC CTA GAC CTT CCA His Leu Val Ile Gly Asn Ser Gly Asp Ser Lys Tyr Leu Asp Leu Pro 620 625 630	2046
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25	ATG AAC ATT TTC TTT GCT CTA CTA GTG AAT GTC AAG GAA GAG GAT GCA Met Asn Ile Phe Ala Leu Leu Val Asn Val Lys Glu Glu Asp Ala 655 660 665	2142
30	AAG GAC TTC ACC AAG TTT ATA AGG GAC ACA ATT GTT CCA AAG CTT GGA Lys Asp Phe Thr Lys Phe Ile Arg Asp Thr Ile Val Pro Lys Leu Gly 670 675 680	2190
35	GCG TGG CCA ACA ATG CAA GAT GTT GCA ACT GCA TGC TAC TTA CTT TCC Ala Trp Pro Thr Met Gln Asp Val Ala Thr Ala Cys Tyr Leu Leu Ser 685 690 695	2238
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45	GAT CAT GAC AAC AAA ACA ATG CAT GTT TTG GAT TCG TAT GGG TCT AGA Asp His Asp Asn Lys Thr Met His Val Leu Asp Ser Tyr Gly Ser Arg 715 720 725 730	2334
50	ACG ACA GGA TAC CAC ATG TTG AAA ATG AAC ACA ACA TCC CAG CTA ATT Thr Thr Gly Tyr His Met Leu Lys Met Asn Thr Ser Gln Leu Ile 735 740 745	2382
55	GAA TTC GTT CAT TCA GGT TTG GAA TCC GAA ATG AAA ACT TAC AAT GTT Glu Phe Val His Ser Gly Leu Glu Ser Glu Met Lys Thr Tyr Asn Val 750 755 760	2430
60	GGA GGG ATG AAC CGA GAT GTG GTC ACA CAA GGT GCA ATT GAG ATG TTG Gly Gly Met Asn Arg Asp Val Val Thr Gln Gly Ala Ile Glu Met Leu 765 770 775	2478
65	ATC AAG TCT ATA TAC AAA CCA CAT CTC ATG AAG CAG TTA CTT GAG GAA Ile Lys Ser Ile Tyr Lys Pro His Leu Met Lys Gln Leu Leu Glu Glu 780 785 790	2526
70	GAG CCA TAC ATA ATT GTC CTG GCA ATA GTC TCC CCT TCA ATT TTA ATT Glu Pro Tyr Ile Ile Val Leu Ala Ile Val Ser Pro Ser Ile Leu Ile 795 800 805 810	2574
75	GCC ATG TAC AAC TCT GGA ACT TTT GAG CAG GCG TTA CAA ATG TGG TTG Ala Met Tyr Asn Ser Gly Thr Phe Glu Gln Ala Leu Gln Met Trp Leu 815 820 825	2622
80	CCA AAT ACA ATG AGG TTA GCT AAC CTC GCT GCC ATC TTG TCA GCC TTA Pro Asn Thr Met Arg Leu Ala Asn Leu Ala Ala Ile Leu Ser Ala Leu 830 835 840	2670

- 33 -

	GAG ATA GCA TAC TTT GAA AAG ATT ATA GCT TTC ATC ACA CTT GTA TTA Glu Ile Ala Tyr Phe Glu Lys Ile Ala Phe Ile Thr Leu Val Leu 1115 1120 1125 1130	3534
5	ATG GCT TTT GAC GCT GAG CGG AGT GAT GGA GTG TTC AAG ATA CTC AAT Met Ala Phe Asp Ala Glu Arg Ser Asp Gly Val Phe Lys Ile Leu Asn 1135 1140 1145	3582
10	AAG TTC AAA GGA ATA CTG AGC TCA ACG GAG AGG GAG ATC ATC TAC ACG Lys Phe Lys Gly Ile Leu Ser Ser Thr Glu Arg Glu Ile Ile Tyr Thr 1150 1155 1160	3630
15	CAG AGT TTG GAT GAT TAC GTT ACA ACC TTT GAT GAC AAT ATG ACA ATC Gln Ser Leu Asp Asp Tyr Val Thr Thr Phe Asp Asp Asn Met Thr Ile 1165 1170 1175	3678
20	AAC CTC GAG TTG AAT ATG GAT GAA CTC CAC AAG ACG AGC CCT CCT GGA Asn Leu Glu Leu Asn Met Asp Glu Leu His Lys Thr Ser Leu Pro Gly 1180 1185 1190	3726
25	GTC ACT TTT AAG CAA TGG TGG AAC AAC CAA ATC ACC CGA GGC AAC GTG Val Thr Phe Lys Gln Trp Trp Asn Asn Gln Ile Ser Arg Gly Asn Val 1195 1200 1205 1210	3774
30	AAG CCA CAT TAT AGA ACT GAG GGG CAC TTC ATG GAG TTT ACC AGA GAT Lys Pro His Tyr Arg Thr Glu Gly His Phe Met Glu Phe Thr Arg Asp 1215 1220 1225	3822
35	ACT GCG GCA TCG GTT GCC AGC GAG ATA TCA CAC TCA CCC GCA AGA GAT Thr Ala Ala Ser Val Ala Ser Glu Ile Ser His Ser Pro Ala Arg Asp 1230 1235 1240	3870
40	TTT CTT GTG AGA GGT GCT GTT GGA TCT GGA AAA TCC ACA GGA CTT CCA Phe Leu Val Arg Gly Ala Val Gly Ser Gly Lys Ser Thr Gly Leu Pro 1245 1250 1255	3918
45	TAC CAT TTA TCA AAG AGA GGG AGA GTG TTA ATG CTT GAG CCT ACC AGA Tyr His Leu Ser Lys Arg Gly Arg Val Leu Met Leu Glu Pro Thr Arg 1260 1265 1270	3966
50	CCA CTC ACA GAT AAC ATG CAC AAG CAA CTG AGA AGT GAA CCA TTT AAC Pro Leu Thr Asp Asn Met His Lys Gln Leu Arg Ser Glu Pro Phe Asn 1275 1280 1285 1290	4014
55	TGC TTC CCA ACT TTG AGG ATG AGA GGG AAG TCA ACT TTT GGG TCA TCA Cys Phe Pro Thr Leu Arg Met Arg Gly Lys Ser Thr Phe Gly Ser Ser 1295 1300 1305	4062
60	CCG ATC ACA GTC ATG ACT AGT GGA TTC GCT TTA CAC CAC TTT GCA CGA Pro Ile Thr Val Met Thr Ser Gly Phe Ala Leu His His Phe Ala Arg 1310 1315 1320	4110
65	AAC ATA GCT GAG GTA AAA ACA TAC GAT TTT GTC ATA ATT GAT GAA TGT Asn Ile Ala Glu Val Lys Thr Tyr Asp Phe Val Ile Ile Asp Glu Cys 1325 1330 1335	4158
70	CAT GTG AAT GAT GCT TCT GCT ATA GCG TTT AGG AAT CTA CTG TTT GAA His Val Asn Asp Ala Ser Ala Ile Ala Phe Arg Asn Leu Leu Phe Glu 1340 1345 1350	4206
75	CAT GAA TTT GAA GGA AAA GTC CTC AAA GTG TCA GCC ACA CCA CCA GGT His Glu Phe Glu Gly Lys Val Leu Lys Val Ser Ala Thr Pro Pro Gly 1355 1360 1365 1370	4254
80	AGA GAA GTT GAA TTT ACA ACT CAG TTT CCC GTG AAA CTC AAG ATA GAA Arg Glu Val Glu Phe Thr Thr Gln Phe Pro Val Lys Leu Lys Ile Glu 1375 1380 1385	4302

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	ATT CCA GAC TCC TTG CAT GAG GAA ATT TGG CAC ATT GTA GTC GCC CAT Ile Pro Asp Ser Leu His Glu Glu Ile Trp His Ile Val Val Ala His 1660 1665 1670	5166
5	AAA GGT GAC TCG GGT ATT GGG AGG CTC ACT AGC GTA CAG GCA GCA AAG Lys Gly Asp Ser Gly Ile Gly Arg Leu Thr Ser Val Gln Ala Ala Lys 1675 1680 1685 1690	5214
10	GTT GTT TAT ACT CTG CAA ACG GAT GTG CAC TCA ATT GCG AGG ACT CTA Val Val Tyr Thr Leu Gln Thr Asp Val His Ser Ile Ala Arg Thr Leu 1695 1700 1705	5262
15	GCA TGC ATC AAT AGA CGC ATA GCA GAT GAA CAA ATG AAG CAG AGT CAT Ala Cys Ile Asn Arg Arg Ile Ala Asp Glu Gln Met Lys Gln Ser His 1710 1715 1720	5310
20	TTT GAA GCC GCA ACT GGG AGA GCA TTT TCC TTC ACA AAT TAC TCA ATA Phe Glu Ala Ala Thr Gly Arg Ala Phe Ser Phe Thr Asn Tyr Ser Ile 1725 1730 1735	5358
25	CAA AGC ATA TTT GAC ACG CTG AAA GCA AAT TAT GCT ACA AAG CAT ACG Gln Ser Ile Phe Asp Thr Leu Lys Ala Asn Tyr Ala Thr Lys His Thr 1740 1745 1750	5406
30	AAA GAA AAT ATT GCA GTG CTT CAG CAG GCA AAA GAT CAA TTG CTA GAG Lys Glu Asn Ile Ala Val Leu Gln Ala Lys Asp Gln Leu Leu Glu 1755 1760 1765 1770	5454
35	TTT TCG AAC CTA GCA AAG GAT CAA GAT GTC ACG GGT ATC ATC CAA GAC Phe Ser Asn Leu Ala Lys Asp Gln Asp Val Thr Gly Ile Ile Gln Asp 1775 1780 1785	5502
40	TTC AAT CAC CTG GAA ACT ATC TAT CTC CAA TCA GAT AGC GAA GTG GCT Phe Asn His Leu Glu Thr Ile Tyr Leu Gln Ser Asp Ser Glu Val Ala 1790 1795 1800	5550
45	AAG CAT CTG AAG CTT AAA AGT CAC TGG AAT AAA AGC CAA ATC ACT AGG Lys His Leu Lys Leu Lys Ser His Trp Asn Lys Ser Gln Ile Thr Arg 1805 1810 1815	5598
50	GAC ATC ATA ATA GCT TTG TCT GTG TTA ATT GGT GGT GGA TGG ATG CTT Asp Ile Ile Ile Ala Leu Ser Val Leu Ile Gly Gly Trp Met Leu 1820 1825 1830	5646
55	GCA ACG TAC TTC AAG GAC AAG TTC AAT GAA CCA GTC TAT TTC CAA GGG Ala Thr Tyr Phe Lys Asp Lys Phe Asn Glu Pro Val Tyr Phe Gln Gly 1835 1840 1845 1850	5694
60	AAG AAG AAT CAG AAG CAC AAG CTT AAG ATG AGA GAG GCG CGT GGG GCT Lys Lys Asn Gln Lys His Lys Leu Lys Met Arg Glu Ala Arg Gly Ala 1855 1860 1865	5742
65	AGA GGG CAA TAT GAG GTT GCA GCG GAG CCA GAG GCG CTA GAA CAT TAC Arg Gly Gln Tyr Glu Val Ala Ala Glu Pro Glu Ala Leu Glu His Tyr 1870 1875 1880	5790
70	TTT GGA AGC GCA TAT AAT AAC AAA GGA AAG CGC AAG GGC ACC ACG AGA Phe Gly Ser Ala Tyr Asn Asn Lys Gly Lys Arg Lys Gly Thr Thr Arg 1885 1890 1895	5838
75	GGA ATG GGT GCA AAG TCT CGG AAA TTC ATA AAC ATG TAT GGG TTT GAT Gly Met Gly Ala Lys Ser Arg Lys Phe Ile Asn Met Tyr Gly Phe Asp 1900 1905 1910	5886
80	CCA ACT GAT TTT TCA TAC ATT AGG TTT GTG GAT CCA TTG ACA GGT CAC Pro Thr Asp Phe Ser Tyr Ile Arg Phe Val Asp Pro Leu Thr Gly His 1915 1920 1925 1930	5934

	ATA CAC TCA GCA TCG AAT TTC ACC AAC ACA AAC AAT TAT TTC ACA AGC Ile His Ser Ala Ser Asn Phe Thr Asn Thr Asn Asn Tyr Phe Thr Ser 2205 2210 2215	6798
5	GTG CCG AAA AAC TTC ATG GAA TTG TTG ACA AAT CAG GAG GCG CAG CAG Val Pro Lys Asn Phe Met Glu Leu Leu Thr Asn Gln Glu Ala Gln Gln 2220 2225 2230	6846
10	TGG GTT AGT GGT TGG CGA TTA AAT GCT GAC TCA GTA TTG TGG GGG GGC Trp Val Ser Gly Trp Arg Leu Asn Ala Asp Ser Val Leu Trp Gly Gly 2235 2240 2245 2250	6894
15	CAT AAA GTT TTC ATG AGC AAA CCT GAA GAG CCT TTT CAG CCA GTT AAG His Lys Val Phe Met Ser Lys Pro Glu Glu Pro Phe Gln Pro Val Lys 2255 2260 2265	6942
20	GAA GCG ACT CAA CTC ATG AAT GAA TTG GTG TAC TCG CAA GGG GAG AAG Glu Ala Thr Gln Leu Met Asn Glu Leu Val Tyr Ser Gln Gly Glu Lys 2270 2275 2280	6990
25	AGG AAA TGG GTC GTG GAA GCA CTG TCA GGG AAC TTG AGG CCA GTG GCT Arg Lys Trp Val Val Glu Ala Leu Ser Gly Asn Leu Arg Pro Val Ala 2285 2290 2295	7038
30	GAG TGT CCC AGT CAG TTA GTC ACA AAG CAT GTG GTT AAA GGA AAG TGT Glu Cys Pro Ser Gln Leu Val Thr Lys His Val Val Lys Gly Lys Cys 2300 2305 2310	7086
35	CCC CTC TTT GAG CTC TAC TTG CAG TTG AAT CCA GAA AAG GAA GCA TAT Pro Leu Phe Glu Leu Tyr Leu Gln Leu Asn Pro Glu Lys Glu Ala Tyr 2315 2320 2325 2330	7134
40	TTT AAA CCG ATG ATG GGA GCA TAT AAG CCA AGT CGA CTT AAT AGA GAG Phe Lys Pro Met Met Gly Ala Tyr Lys Pro Ser Arg Leu Asn Arg Glu 2335 2340 2345	7182
45	GCG TTC CTC AAG GAC ATT CTA AAA TAT GCT AGT GAA ATT GAG ATT GGG Ala Phe Leu Lys Asp Ile Leu Lys Tyr Ala Ser Glu Ile Glu Ile Gly 2350 2355 2360	7230
50	AAT GTG GAT TGT GAC TTG CTG GAG CTT GCA ATA AGC ATG CTC GTC ACA Asn Val Asp Cys Asp Leu Leu Glu Leu Ala Ile Ser Met Leu Val Thr 2365 2370 2375	7278
55	AAG CTC AAG GCG TTA GGA TTC CCA ACT GTG AAC TAC ATC ACT GAC CCA Lys Leu Lys Ala Leu Gly Phe Pro Thr Val Asn Tyr Ile Thr Asp Pro 2380 2385 2390	7326
60	GAG GAA ATT TTT AGT GCA TTG AAT ATG AAA GCA GCT ATG GGA GCA CTA Glu Glu Ile Phe Ser Ala Leu Asn Met Lys Ala Ala Met Gly Ala Leu 2395 2400 2405 2410	7374
65	TAC AAA GGC AAG AAG AAA GAA GCT CTC AGC GAG CTC ACA CTA GAT GAG Tyr Lys Gly Lys Lys Glu Ala Leu Ser Glu Leu Thr Leu Asp Glu 2415 2420 2425	7422
70	CAG GAG GCA ATG CTC AAA GCA AGT TGC CTG CGA CTG TAT ACG GGA AAG Gln Glu Ala Met Leu Lys Ala Ser Cys Leu Arg Leu Tyr Thr Gly Lys 2430 2435 2440	7470
75	TTG GGA ATT TGG AAT GGC TCA TTG AAA GCA GAG TTG CGT CCA ATT GAG Leu Gly Ile Trp Asn Gly Ser Leu Lys Ala Glu Leu Arg Pro Ile Glu 2445 2450 2455	7518
80	AAG GTT GAA AAC AAC AAA ACG CGA ACT TTC ACA GCA GCA CCA ATA GAC Lys Val Glu Asn Asn Lys Thr Arg Thr Phe Thr Ala Ala Pro Ile Asp 2460 2465 2470	7566

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	AAG GCG CCA TAT CTG GCT GAG ACT GCG CTT AAG TTT TTG TAC ACA TCT Lys Ala Pro Tyr Leu Ala Glu Thr Ala Leu Lys Phe Leu Tyr Thr Ser 2750 2755 2760	8430
5	CAG CAC GGA ACA AAC TCT GAG ATA GAA GAG TAT TTA AAA GTG TTG TAT Gln His Gly Thr Asn Ser Glu Ile Glu Glu Tyr Leu Lys Val Leu Tyr 2765 2770 2775	8478
10	GAT TAC GAT ATT CCA ACG ACT GAG AAT CTT TAT TTT CAG AGT GGC ACT Asp Tyr Asp Ile Pro Thr Thr Glu Asn Leu Tyr Phe Gln Ser Gly Thr 2780 2785 2790	8526
15	GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Asp Gln Lys Asp Asp 2795 2800 2805 2810	8574
	AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr 2815 2820 2825	8622
20	TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu 2830 2835 2840	8670
25	CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT Gln Tyr Pro Arg Met Arg Gly Glu Val Val Val Asn Leu Asn His Leu 2845 2850 2855	8718
30	TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr 2860 2865 2870	8766
35	CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly 2875 2880 2885 2890	8814
	GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp 2895 2900 2905	8862
40	TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met 2910 2915 2920	8910
45	ATG GAT GGT GAG GAT CAA GTT TCA TAC CCG CTG AAA CCA ATG GTT GAA Met Asp Gly Glu Asp Gln Val Ser Tyr Pro Leu Lys Pro Met Val Glu 2925 2930 2935	8958
50	AAC GCG CAG CCA ACA CTG AGG CAA ATT ATG ACA CAC TTC AGT GAC CTG Asn Ala Gln Pro Thr Leu Arg Gln Ile Met Thr His Phe Ser Asp Leu 2940 2945 2950	9006
55	GCT GAA GCG TAT ATT GAG ATG AGG AAT AGG GAG CGA CCA TAC ATG CCT Ala Glu Ala Tyr Ile Glu Met Arg Asn Arg Glu Arg Pro Tyr Met Pro 2955 2960 2965 2970	9054
	AGG TAT GGT CTA CAG AGA AAC ATT ACA GAC ATG AGT TTG TCA CGC TAT Arg Tyr Gly Leu Gln Arg Asn Ile Thr Asp Met Ser Leu Ser Arg Tyr 2975 2980 2985	9102
60	GCG TTC GAC TTC TAT GAG CTA ACT TCA AAA ACA CCT GTT AGA GCG AGG Ala Phe Asp Phe Tyr Glu Leu Thr Ser Lys Thr Pro Val Arg Ala Arg 2990 2995 3000	9150
65	GAG GCG CAT ATG CAA ATG AAA GCT GCT GCA GTA CGA AAC AGT GGA ACT Glu Ala His Met Gln Met Lys Ala Ala Ala Val Arg Asn Ser Gly Thr 3005 3010 3015	9198

## (x) PUBLICATION INFORMATION:

5

(A) AUTHORS: Allison et al.

(B) TITLE: The nucleotide sequence of the coding region of Tobacco Etch Virus Genomic RNA: Evidence for the Synthesis of a Single Polyprotein

10 (C) JOURNAL: Virology

(D) VOLUME: 154

(E) ISSUE: --

(F) PAGES: 9-20

15

(A) AUTHORS: Lindbo and Dougherty

(B) TITLE: Untranslatable Transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in Transgenic Plants and Protoplasts

20 (C) JOURNAL: Virology

(D) VOLUME: 189

(E) ISSUE: --

(F) PAGES: 725-733

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

	ATG GGC ACT Met Gly Thr	9
25	1	
	GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT	
	Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp	
	5                    10                    15	
30		
	AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT	
	Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr	
	20                25                30                35	
35		
	TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT	
	Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu	
	40                45                50	
40		
	CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT	
	Gln Tyr Pro Arg Met Arg Gly Glu Val Val Val Asn Leu Asn His Leu	
	55                60                65	
45		
	TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA	
	Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr	
	70                75                80	
50		
	CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA	
	His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly	
	85                90                95	
55		
	GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG	
	Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp	
	100              105              110              115	
60		
	TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG	
	Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met	
	120              125              130	

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## (ix) FEATURE:

5 (A) NAME/KEY: Mutation of AGT-GGC (Ser-Gly) to ATG-GCC (Met-Ser)

10 (B) LOCATION: Nucleotides 1-6 of SEQ ID NO. 3 (corresponding to nucleotides 8518-8523 of SEQ ID NO. 1)

15 (A) NAME/KEY: Frameshift mutation (insertion of T) producing stop codon

(B) LOCATION: Nucleotide 13 of SEQ ID No. 3 (corresponding to position between nucleotides 8529 and 8530 of SEQ. ID No. 1)

(D) OTHER INFORMATION: SEQ ID NO: 3 is the modified Tobacco Etch Virus coat protein gene present in pTC:RC.

## (x) PUBLICATION INFORMATION:

20 (A) AUTHORS: J. A. Lindbo and W. G. Dougherty

25 (B) TITLE: Pathogen-Derived Resistance to a Potyvirus: Immune and Resistant Phenotypes in Transgenic Tobacco Expressing Altered Forms of a Potyvirus Coat Protein Nucleotide Sequence

(C) JOURNAL: Molecular Plant-Microbe Interactions

(D) VOLUME: 5

(E) ISSUE: 2

(F) PAGES: 144-153

30 (A) AUTHORS: J. A. Lindbo and W. G. Dougherty

35 (B) TITLE: Untranslatable Transcripts of the Tobacco Etch Virus Coat Protein Gene Sequence Can Interfere with Tobacco Etch Virus Replication in Transgenic Plants and Protoplasts

(C) JOURNAL: Virology

(D) VOLUME: 189

40 (E) ISSUE: --

(F) PAGES: 725-733

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATG GCC ACT  
Met Ser Thr

9

45 GTG TGA TGA TGGTGCTAGC GCTGGTAAGA AGAAAGATCA AAAGGATGAT  
Val

58

- 45 -

(D) OTHER INFORMATION: SEQ ID No. 4 is the modified Tobacco Etch Virus Coat protein gene present in pTC:AS. It is the inverse complement of SEQ ID No. 2.

5 (x)

## PUBLICATION INFORMATION:

(A) AUTHORS: J. A. Lindbo and  
W. G. Dougherty

10

(B) TITLE: Untranslatable Transcripts of the Tobacco Etch Virus Coat Protein Gene Sequence Can Interfere with Tobacco Etch Virus Replication in Transgenic Plants and Protoplasts

15

(C) JOURNAL: Virology

(D) VOLUME: 189

(E) ISSUE: --

(F) PAGES: 725-733

20

(A) AUTHORS: J. A. Lindbo and  
W. G. Dougherty

25

(B) TITLE: Pathogen-Derived Resistance to a Potyvirus: Immune and Resistant Phenotypes in Transgenic Tobacco Expressing Altered Forms of a Potyvirus Coat Protein Nucleotide Sequence

(C) JOURNAL: Molecular Plant-Microbe Interactions

(D) VOLUME: 5

(E) ISSUE: 2

(F) PAGES: 144-153

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCACTGGCGG ACCCCTAATA GTGTGTGCAT	60
TTACGGTTTC ACATCGTCCG CTGTGTGCCG	
TTCAGTGTCT TCCCTGCGAG TACCCACGTT GCCATCAAGA CCAAATAACC TAGTTCCACT	120
GTTCGTACT GCAGCAGCTT TCATTTGCAT ATGCCGCCTCC CTCGCTCTAA CAGGTGT----	180
TGAAGTTAGC TCATAGAACG CGAACGGATA GCGTGACAAA CTCATGTCTG TAATGTTCT	240
CTGTAGACCA TACCTAGGCA TGTATGGTCG CTCCCTATTG CTCATCTCAA TATAAGCTTC	300
AGCCAGGTCA CTGAAGTGTG TCATAATTTC CCTCAGTGTG GGCTGCCGT TTTCAACCAT	360
TGGTTTCAGC GGGTATGAAA CTTGATCCTC ACCATCCATC ATAACCCAAAG TTCCGTTCAA	420
ATTTGGGAA GTCCCCATTCT CTATGCCACCA CACCATAAAT CCATTTAGCA ATATTTTCAT	480
TTGCTCTTCA TTCACTCCAT AGGCTGTCACT CACTGCCTCA TGCCACGGGG CAAACTGCTC	540
ATGTGTGGCT CGAGCATTTC ACRAATCAAT TTGCTGTGGC TTGTATCCTA AAAGGTGATT	600
CAAGTTTACA ACCACCTCTC CCCTCTATCT TGGATATTGA AGTTTTGTGG CCATAGCATT	660
TATTCCTGGA ACTGAGAATG TTCTGAAAGT TCCAGGATTA ACATCCCTAT CCTTTGAGC	720
CTGCTCAGGG ACTTTTATCAT CCTTTTGATC TTTCTCTTA CCAGGCTCAG CACCAACATC	780
CACAGTGCCC AT	792

15. A differentiated plant comprising transformed plant cells of claim 10.

16. A differentiated plant comprising transformed plant cells of claim 11.

5 17. A differentiated plant comprising transformed plant cells of claim 12.

18. A recombinant gene comprising: control regions which regulate transcription of the gene; and

10 a region, derived from a plant virus, mutated so as to render the RNA transcribed from the gene untranslatable.

19. The recombinant gene of claim 18 wherein the plant virus is a potyvirus.

15 20. The recombinant gene of claim 19 wherein the virus-derived region is derived from the region of the viral genome encoding a coat protein.

21. The recombinant gene of claim 20 wherein the potyvirus is Tobacco Etch Virus.

20 22. A method of producing plants with a reduced susceptibility to viral infection, comprising:

forming a recombinant gene derived, in part, from viral RNA wherein the gene is mutated to encode an untranslatable plus sense RNA molecule; and

25 transforming plants with the recombinant gene.

23. The method of claim 22 wherein the method of producing plants comprises:

constructing a recombinant gene comprising a region of a viral genome capable of being transcribed in a plant;

mutating the recombinant gene to encode an untranslatable plus sense RNA molecule;

cloning the recombinant untranslatable gene into a plant transformation vector;

transforming plant cells with the transformation vector; and

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NAAATAACAA ATCTAACAC AACATATACA AAACAAACGA ATCTCAAGCA ATCAAGCATT	60
CTACTTCTAT TGCAGCAATT TAAATCATTT CTTTAAAGC AAAAGCAATT TTCTGAAAAT	120
TTTCACCATT TACGAACCGAT AGCA ATG GCA CTG ATC TTT GGC ACA GTC AAC GCT Met Ala Leu Ile Phe Gly Thr Val Asn Ala	174
1 5 10	
AAC ATC CTG AAG GAA GTG TTC GGT GGA GCT CGT ATG GCT TGC GTT ACC Asn Ile Leu Lys Glu Val Phe Gly Ala Arg Met Ala Cys Val Thr	222
15 20 25	
AGC GCA CAT ATG GCT GGA GCG AAT GGA AGC ATT TTG AAG AAG GCA GAA Ser Ala His Met Ala Gly Ala Asn Gly Ser Ile Leu Lys Lys Ala Glu	270
30 35 40	
GAG ACC TCT CGT GCA ATC ATG CAC AAA CCA GTG ATC TTC GGA GAA GAC Glu Thr Ser Arg Ala Ile Met His Lys Pro Val Ile Phe Gly Glu Asp	318
45 50 55	
TAC ATT ACC GAG GCA GAC TTG CCT TAC ACA CCA CTC CAT TTA GAG GTC Tyr Ile Thr Glu Ala Asp Leu Pro Tyr Thr Pro Leu His Leu Glu Val	366
60 65 70	
GAT GCT GAA ATG GAG CGG ATG TAT TAT CTT GGT CGT CGC GCG CTC ACC Asp Ala Glu Met Glu Arg Met Tyr Tyr Leu Gly Arg Arg Ala Leu Thr	414
75 80 85 90	
CAT GCC AAG AGA CGC AAA GTT TCT GTG AAT AAC AAG AGG AAC AGG AGA His Gly Lys Arg Arg Lys Val Ser Val Asn Asn Lys Arg Asn Arg Arg	462
95 100 105	
AGG AAA GTG GCC AAA ACG TAC GTG GGG CGT GAT TCC ATT GTT GAG AAG Arg Lys Val Ala Lys Thr Tyr Val Gly Arg Asp Ser Ile Val Glu Lys	510
110 115 120	
ATT GTA GTG CCC CAC ACC GAG AGA AAG GTT GAT ACC ACA GCA GCA GTG Ile Val Val Pro His Thr Glu Arg Lys Val Asp Thr Thr Ala Ala Val	558
125 130 135	
GAA GAC ATT TGC AAT GAA GCT ACC ACT CAA CTT GTG CAT AAT AGT ATG Glu Asp Ile Cys Asn Glu Ala Thr Thr Gln Leu Val His Asn Ser Met	606
140 145 150	
CCA AAG CGT AAG AAG CAG AAA AAC TTC TTG CCC GCC ACT TCA CTA AGT Pro Lys Arg Lys Lys Gln Lys Asn Phe Leu Pro Ala Thr Ser Leu Ser	654
155 160 165 170	
AAC GTG TAT GCC CAA ACT TGG AGC ATA GTG CGC AAA CGC CAT ATG CAG Asn Val Tyr Ala Gln Thr Trp Ser Ile Val Arg Lys Arg His Met Gln	702
175 180 185	
GTG GAG ATC ATT AGC AAG AAG AGC GTC CGA GCG AGG GTC AAG AGA TTT Val Glu Ile Ile Ser Lys Lys Ser Val Arg Ala Arg Val Lys Arg Phe	750
190 195 200	
GAG GGC TCG GTG CAA TTG TTC GCA AGT GTG CGT CAC ATG TAT GGC GAG Glu Gly Ser Val Gln Leu Phe Ala Ser Val Arg His Met Tyr Gly Glu	798
205 210 215	
AGG AAA AGG GTG GAC TTA CGT ATT GAC AAC TGG CAG CAA GAG ACA CTT Arg Lys Arg Val Asp Leu Arg Ile Asp Asn Trp Gln Gln Glu Thr Leu	846
220 225 230	

FIG. 1

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CTA GAC CTT GCT AAA AGA TTT AAG AAT GAG AGA GTG GAT CAA TCG AAG Leu Asp Leu Ala Lys Arg Phe Lys Asn Glu Arg Val Asp Gln Ser Lys 235 240 245 250	894
CTC ACT TTT GGT TCA AGT GGC CTA GTT TTG AGG CAA GGC TCG TAC GGA Leu Thr Phe Gly Ser Ser Gly Leu Val Leu Arg Gln Gly Ser Tyr Gly 255 260 265	942
CCT GCG CAT TGG TAT CGA CAT GGT ATG TTC ATT GTA CGC GGT CGG TCG Pro Ala His Trp Tyr Arg His Gly Met Phe Ile Val Arg Gly Arg Ser 270 275 280	990
GAT GGG ATG TTG GTG GAT GCT CGT GCG AAG GTA ACG TTC GCT GTT TGT Asp Gly Met Leu Val Asp Ala Arg Ala Lys Val Thr Phe Ala Val Cys 285 290 295	1038
CAC TCA ATG ACA CAT TAT AGC GAC AAA TCA ATC TCT GAG GCA TTC TTC His Ser Met Thr His Tyr Ser Asp Lys Ser Ile Ser Glu Ala Phe Phe 300 305 310	1086
ATA CCA TAC TCT AAG AAA TTC TTG GAG TTG AGA CCA GAT GGA ATC TCC Ile Pro Tyr Ser Lys Lys Phe Leu Glu Leu Arg Pro Asp Gly Ile Ser 315 320 325 330	1134
CAT GAG TGT ACA AGA GGA GTA TCA GTT GAG CGG TGC GGT GAG GTG GCT His Glu Cys Thr Arg Gly Val Ser Val Glu Arg Cys Gly Glu Val Ala 335 340 345	1182
GCA ATC CTG ACA CAA GCA CTT TCA CCG TGT GGT AAG ATC ACA TGC AAA Ala Ile Leu Thr Gln Ala Leu Ser Pro Cys Gly Lys Ile Thr Cys Lys 350 355 360	1230
CGT TGC ATG GTT GAA ACA CCT GAC ATT GTT GAG GGT GAG TCG GGA GAA Arg Cys Met Val Glu Thr Pro Asp Ile Val Glu Gly Glu Ser Gly Glu 365 370 375	1278
AGT GTC ACC AAC CAA GGT AAG CTC CTA GCA ATG CTG AAA GAA CAG TAT Ser Val Thr Asn Gln Gly Lys Leu Leu Ala Met Leu Lys Glu Gln Tyr 380 385 390	1326
CCA GAT TTC CCA ATG GCC GAG AAA CTA CTC ACA AGG TTT TTG CAA CAG Pro Asp Phe Pro Met Ala Glu Lys Leu Leu Thr Arg Phe Leu Gln Gln 395 400 405 410	1374
AAA TCA CTA GTA AAT ACA AAT TTG ACA GCC TGC GTG AGC GTC AAA CAA Lys Ser Leu Val Asn Thr Asn Leu Thr Ala Cys Val Ser Val Lys Gln 415 420 425	1422
CTC ATT GGT GAC CGC AAA CAA GCT CCA TTC ACA CAC GTC CTG GCT GTC Leu Ile Gly Asp Arg Lys Gln Ala Pro Phe Thr His Val Leu Ala Val 430 435 440	1470
AGC GAA ATT CTG TTT AAA GCC AAT AAA CTA ACA GGG GCT GAT CTC GAA Ser Glu Ile Leu Phe Lys Gly Asn Lys Leu Thr Gly Ala Asp Leu Glu 445 450 455	1518
GAG GCA AGC ACA CAT ATG CTT GAA ATA GCA AGG TTC TTG AAC AAT CGC Glu Ala Ser Thr His Met Leu Glu Ile Ala Arg Phe Leu Asn Asn Arg 460 465 470	1566
ACT GAA AAT ATG CGC ATT GGC CAC CTT GGT TCT TTC AGA AAT AAA ATC Thr Glu Asn Met Arg Ile Gly His Leu Gly Ser Phe Arg Asn Lys Ile 475 480 485 490	1614

FIG. 1

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TCA TCG AAG GCC CAT GTG AAT AAC GCA CTC ATG TGT GAT AAT CAA CTT Ser Ser Lys Ala His Val Asn Asn Ala Leu Met Cys Asp Asn Gln Leu 495 500 505	1662
GAT CAG AAT GGG AAT TTT ATT TGG GGA CTA AGG GGT GCA CAC GCA AAG Asp Gln Asn Gly Asn Phe Ile Trp Gly Leu Arg Gly Ala His Ala Lys 510 515 520	1710
AGG TTT CTT AAA GGA TTT TTC ACT GAG ATT GAC CCA AAT GAA GGA TAC Arg Phe Leu Lys Gly Phe Phe Thr Glu Ile Asp Pro Asn Glu Gly Tyr 525 530 535	1758
GAT AAG TAT GTT ATC AGG AAA CAT ATC AGG GGT AGC AGA AAG CTA GCA Asp Lys Tyr Val Ile Arg Lys His Ile Arg Gly Ser Arg Lys Leu Ala 540 545 550	1806
ATT GGC AAT TTG ATA ATG TCA ACT GAC TTC CAG ACG CTC AGG CAA CAA Ile Gly Asn Leu Ile Met Ser Thr Asp Phe Gln Thr Leu Arg Gln Gln 555 560 565 570	1854
ATT CAA GGC GAA ACT ATT GAG CGT AAA GAA ATT GGG AAT CAC TGC ATT Ile Gln Gly Glu Thr Ile Glu Arg Lys Glu Ile Gly Asn His Cys Ile 575 580 585	1902
TCA ATG CGG AAT GGT AAT TAC GTG TAC CCA TGT TGT TGT GTT ACT CTT Ser Met Arg Asn Gly Asn Tyr Val Tyr Pro Cys Cys Cys Val Thr Leu 590 595 600	1950
GAA GAT GGT AAG GCT CAA TAT TCG GAT CTA AAG CAC CCA ACG AAG AGA Glu Asp Gly Lys Ala Gln Tyr Ser Asp Leu Lys His Pro Thr Lys Arg 605 610 615	1998
CAT CTG GTC ATT GGC AAC TCT GGC GAT TCA AAG TAC CTA GAC CTT CCA His Leu Val Ile Gly Asn Ser Gly Asp Ser Lys Tyr Leu Asp Leu Pro 620 625 630	2046
GTT CTC AAT GAA GAG AAA ATG TAT ATA GCT AAT GAA GGT TAT TGC TAC Val Leu Asn Glu Glu Lys Met Tyr Ile Ala Asn Glu Gly Tyr Cys Tyr 635 640 645 650	2094
ATG AAC ATT TTC TTT GCT CTA CTA GTG AAT GTC AAG GAA GAG GAT GCA Met Asn Ile Phe Phe Ala Leu Leu Val Asn Val Lys Glu Asp Ala 655 660 665	2142
AAG GAC TTC ACC AAG TTT ATA AGG GAC ACA ATT GTT CCA AAG CTT GGA Lys Asp Phe Thr Lys Phe Ile Arg Asp Thr Ile Val Pro Lys Leu Gly 670 675 680	2190
GCG TGG CCA ACA ATG CAA GAT GTT GCA ACT GCA TGC TAC TTA CTT TCC Ala Trp Pro Thr Met Gln Asp Val Ala Thr Ala Cys Tyr Leu Leu Ser 685 690 695	2238
ATT CTT TAC CCA GAT GTC CTG AGA GCT GAA CTA CCC AGA ATT TTG GTT Ile Leu Tyr Pro Asp Val Leu Arg Ala Glu Leu Pro Arg Ile Leu Val 700 705 710	2286
GAT CAT GAC AAC AAA ACA ATG CAT GTT TTG GAT TCG TAT GGG TCT AGA Asp His Asp Asn Lys Thr Met His Val Leu Asp Ser Tyr Gly Ser Arg 715 720 725 730	2334
ACG ACA GGA TAC CAC ATG TTG AAA ATG AAC ACA ACA TCC CAG CTA ATT Thr Thr Gly Tyr His Met Leu Lys Met Asn Thr Thr Ser Gln Leu Ile 735 740 745	2382

FIG. 1

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GAA TTC GTT CAT TCA GGT TTG GAA TCC GAA ATG AAA ACT TAC AAT GTT Glu Phe Val His Ser Gly Leu Glu Ser Glu Met Lys Thr Tyr Asn Val 750 755 760	2430
GGA GGG ATG AAC CGA GAT GTG GTC ACA CAA GGT GCA ATT GAG ATG TTG Gly Gly Met Asn Arg Asp Val Val Thr Gln Gly Ala Ile Glu Met Leu 765 770 775	2478
ATC AAG TCT ATA TAC AAA CCA CAT CTC ATG AAG CAG TTA CTT GAG GAA Ile Lys Ser Ile Tyr Lys Pro His Leu Met Lys Gln Leu Leu Glu Glu 780 785 790	2526
GAG CCA TAC ATA ATT GTC CTG GCA ATA GTC TCC CCT TCA ATT TTA ATT Glu Pro Tyr Ile Ile Val Leu Ala Ile Val Ser Pro Ser Ile Leu Ile 795 800 805 810	2574
GCC ATG TAC AAC TCT GGA ACT TTT GAG CAG GCG TTA CAA ATG TGG TTG Ala Met Tyr Asn Ser Gly Thr Phe Glu Gln Ala Leu Gln Met Trp Leu 815 820 825	2622
CCA AAT ACA ATG AGG TTA GCT AAC CTC GCT GCC ATC TTG TCA GCC TTA Pro Asn Thr Met Arg Leu Ala Asn Leu Ala Ala Ile Leu Ser Ala Leu 830 835 840	2670
GCG CAA AAG TTA ACT TTG GCA GAT TTG TTC GTC CAG CAG CGT AAT TTG Ala Gln Lys Leu Thr Leu Ala Asp Leu Phe Val Gln Gln Arg Asn Leu 845 850 855	2718
ATT AAT GAG TAT GCG CAG GTA ATT TTG GAC AAT CTG ATT GAC GGT GTC Ile Asn Glu Tyr Ala Gln Val Ile Leu Asp Asn Leu Ile Asp Gly Val 860 865 870	2766
AGG GTT AAT CAT TCG CTA TCC CTA GCA ATG GAA ATT GTT ACT ATT AAG Arg Val Asn His Ser Leu Ser Leu Ala Met Glu Ile Val Thr Ile Lys 875 880 885 890	2814
CTG GCC ACC CAA GAG ATG GAC ATG GCG TTG AGG GAA GGT GGC TAT GCT Leu Ala Thr Gln Glu Met Asp Met Ala Leu Arg Glu Gly Gly Tyr Ala 895 900 905	2862
GTG ACC TCT GAA AAG GTG CAT GAA ATG TTG GAA AAA AAC TAT GTA AAG Val Thr Ser Glu Lys Val His Glu Met Leu Glu Lys Asn Tyr Val Lys 910 915 920	2910
GCT TTG AAG GAT GCA TGG GAC GAA TTA ACT TTG TTG GAA AAA TTC TCC Ala Leu Lys Asp Ala Trp Asp Glu Leu Thr Trp Leu Glu Lys Phe Ser 925 930 935	2958
GCA ATC AGG CAT TCA AGA AAG CTC TTG AAA TTT GGG CGA AAG CCT TTA Ala Ile Arg His Ser Arg Lys Leu Leu Lys Phe Gly Arg Lys Pro Leu 940 945 950	3006
ATC ATG AAA AAC ACC GTA GAT TGC GGC GGA CAT ATA GAC TTG TCT GTG Ile Met Lys Asn Thr Val Asp Cys Gly Gly His Ile Asp Leu Ser Val 955 960 965 970	3054
AAA TCG CTT TTC AAG TTC CAC TTG GAA CTC CTG AAG GGA ACC ATC TCA Lys Ser Leu Phe Lys Phe His Leu Glu Leu Leu Lys Gly Thr Ile Ser 975 980 985	3102
AGA GCC GTA AAT GGT GGC GCA AGA AAG GTA AGA GTA GCG AAG AAT GCC Arg Ala Val Asn Gly Gly Ala Arg Lys Val Arg Val Ala Lys Asn Ala 990 995 1000	3150

FIG. 1

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ATG ACA AAA GGG GTT TTT CTC AAA ATC TAC AGC ATG CTT CCT GAC GTC Met Thr Lys Gly Val Phe Leu Lys Ile Tyr Ser Met Leu Pro Asp Val 1005 1010 1015	3198
TAC AAG TTT ATC ACA GTC TCG AGT GTC CTT TCC TTG TTG ACA TTC Tyr Lys Phe Ile Thr Val Ser Ser Val Leu Ser Leu Leu Thr Phe 1020 1025 1030	3246
TTA TTT CAA ATT GAC TGC ATG ATA AGG GCA CAC CGA GAG GCG AAG GTT Leu Phe Gln Ile Asp Cys Met Ile Arg Ala His Arg Glu Ala Lys Val 1035 1040 1045 1050	3294
GCT GCA CAG TTG CAG AAA GAG AGC GAG TGG GAC AAT ATC ATC AAT AGA Ala Ala Gln Leu Gln Lys Glu Ser Glu Trp Asp Asn Ile Ile Asn Arg 1055 1060 1065	3342
ACT TTC CAG TAT TCT AAG CTT GAA AAT CCT ATT GGC TAT CGC TCT ACA Thr Phe Gln Tyr Ser Lys Leu Glu Asn Pro Ile Gly Tyr Arg Ser Thr 1070 1075 1080	3390
GCG GAG GAA AGA CTC CAA TCA GAA CAC CCC GAG GCT TTC GAG TAC TAC Ala Glu Glu Arg Leu Gln Ser Glu His Pro Glu Ala Phe Glu Tyr Tyr 1085 1090 1095	3438
AAG TTT TGC ATT GGA AAG GAA GAC CTC GTT GAA CAG GCA AAA CAA CCG Lys Phe Cys Ile Gly Lys Glu Asp Leu Val Glu Gln Ala Lys Gln Pro 1100 1105 1110	3486
GAG ATA GCA TAC TTT GAA AAG ATT ATA GCT TTC ATC ACA CTT GTA TTA Glu Ile Ala Tyr Phe Glu Lys Ile Ile Ala Phe Ile Thr Leu Val Leu 1115 1120 1125 1130	3534
ATG GCT TTT GAC GCT GAG CGG AGT GAT GGA GTG TTC AAG ATA CTC AAT Met Ala Phe Asp Ala Glu Arg Ser Asp Gly Val Phe Lys Ile Leu Asn 1135 1140 1145	3582
AAG TTC AAA GGA ATA CTG AGC TCA ACG GAG AGG GAG ATC ATC TAC ACG Lys Phe Lys Gly Ile Leu Ser Ser Thr Glu Arg Glu Ile Ile Tyr Thr 1150 1155 1160	3630
CAG AGT TTG GAT GAT TAC GTT ACA ACC TTT GAT GAC AAT ATG ACA ATC Gln Ser Leu Asp Asp Tyr Val Thr Thr Phe Asp Asp Asn Met Thr Ile 1165 1170 1175	3678
AAC CTC GAG TTG AAT ATG GAT GAA CTC CAC AAG ACG AGC CTT CCT GGA Asn Leu Glu Leu Asn Met Asp Glu Leu His Lys Thr Ser Leu Pro Gly 1180 1185 1190	3726
GTC ACT TTT AAG CAA TGG TGG AAC AAC CAA ATC AGC CGA GGC AAC GTG Val Thr Phe Lys Gln Trp Trp Asn Asn Gln Ile Ser Arg Gly Asn Val 1195 1200 1205 1210	3774
AAG CCA CAT TAT AGA ACT GAG GGG CAC TTC ATG GAG TTT ACC AGA GAT Lys Pro His Tyr Arg Thr Glu Gly His Phe Met Glu Phe Thr Arg Asp 1215 1220 1225	3822
ACT GCG GCA TCG GTT GCC AGC GAG ATA TCA CAC TCA CCC GCA AGA GAT Thr Ala Ala Ser Val Ala Ser Glu Ile Ser His Ser Pro Ala Arg Asp 1230 1235 1240	3870
TTT CTT GTG AGA GGT GCT GTT GGA TCT GGA AAA TCC ACA GGA CTT CCA Phe Leu Val Arg Gly Ala Val Gly Ser Gly Lys Ser Thr Gly Leu Pro 1245 1250 1255	3918

FIG. 1

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TAC CAT TTA TCA AAG AGA GGG AGA GTG TTA ATG CTT GAG CCT ACC AGA Tyr His Leu Ser Lys Arg Gly Arg Val Leu Met Leu Glu Pro Thr Arg 1260 1265 1270	3966
CCA CTC ACA GAT AAC ATG CAC AAG CAA CTG AGA AGT GAA CCA TTT AAC Pro Leu Thr Asp Asn Met His Lys Gln Leu Arg Ser Glu Pro Phe Asn 1275 1280 1285 1290	4014
TGC TTC CCA ACT TTG AGG ATG AGA GGG AAG TCA ACT TTT GGG TCA TCA Cys Phe Pro Thr Leu Arg Met Arg Gly Lys Ser Thr Phe Gly Ser Ser 1295 1300 1305	4062
CCG ATC ACA GTC ATG ACT AGT GGA TTC GCT TTA CAC CAC TTT GCA CGA Pro Ile Thr Val Met Thr Ser Gly Phe Ala Leu His His Phe Ala Arg 1310 1315 1320	4110
AAC ATA GCT GAG GTA AAA ACA TAC GAT TTT GTC ATA ATT GAT GAA TGT Asn Ile Ala Glu Val Lys Thr Tyr Asp Phe Val Ile Ile Asp Glu Cys 1325 1330 1335	4158
CAT GTG AAT GAT GCT TCT GCT ATA GCG TTT AGG AAT CTA CTG TTT GAA His Val Asn Asp Ala Ser Ala Ile Ala Phe Arg Asn Leu Leu Phe Glu 1340 1345 1350	4206
CAT GAA TTT GAA GGA AAA GTC CTC AAA GTG TCA GCC ACA CCA CCA GGT His Glu Phe Glu Gly Lys Val Leu Lys Val Ser Ala Thr Pro Pro Gly 1355 1360 1365 1370	4254
AGA GAA GTT GAA TTT ACA ACT CAG TTT CCC GTG AAA CTC AAG ATA GAA Arg Glu Val Glu Phe Thr Thr Gln Phe Pro Val Lys Leu Lys Ile Glu 1375 1380 1385	4302
GAG GCT CTT AGC TTT CAG GAA TTT GTA AGT TTA CAA GGG ACA GGT GCC Glu Ala Leu Ser Phe Gln Glu Phe Val Ser Leu Gln Gly Thr Gly Ala 1390 1395 1400	4350
AAC GCC GAT GTG ATT AGT TGT GGC GAC AAC ATA CTA GTA TAT GTT GCT Asn Ala Asp Val Ile Ser Cys Gly Asp Asn Ile Leu Val Tyr Val Ala 1405 1410 1415	4398
AGC TAC AAT GAT GTT GAT AGT CTT GGC AAG CTC CTT GTG CAA AAG GGA Ser Tyr Asn Asp Val Asp Ser Leu Gly Lys Leu Leu Val Gln Lys Gly 1420 1425 1430	4446
TAC AAA GTG TCG AAG ATT GAT GGA AGA ACA ATG AAG AGT GGA GGA ACT Tyr Lys Val Ser Lys Ile Asp Gly Arg Thr Met Lys Ser Gly Gly Thr 1435 1440 1445 1450	4494
GAA ATA ATC ACT GAA GGT ACT TCA GTG AAA AAG CAT TTC ATA GTC GCA Glu Ile Ile Thr Glu Gly Thr Ser Val Lys Lys His Phe Ile Val Ala 1455 1460 1465	4542
ACT AAC ATT ATT GAG AAT GGT GTA ACC ATT GAC ATT GAT GTA GTT GTG Thr Asn Ile Ile Glu Asn Gly Val Thr Ile Asp Ile Asp Val Val Val 1470 1475 1480	4590
GAT TTT GGG ACT AAG GTT GTA CCA GTT TTG GAT GTG GAC AAT AGA GCG Asp Phe Gly Thr Lys Val Val Pro Val Leu Asp Val Asp Asn Arg Ala 1481 1490 1495	4638
GTG CAG TAC AAC AAA ACT GTG GTG AGT TAT GGG GAG CGC ATC CAA AAA Val Gln Tyr Asn Lys Thr Val Val Ser Tyr Gly Glu Arg Ile Gln Lys 1500 1505 1510	4686

FIG. 1

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CTC GGT AGA GTT GGG CGA CAC AAG GAA GGA GTA GCA CTT CGA ATT GGC Leu Gly Arg Val Gly Arg His Lys Glu Gly Val Ala Leu Arg Ile Gly 1515 1520 1525 1530	4734
CAA ACA AAT AAA ACA CTG GTT GAA ATT CCA GAA ATG GTT GCC ACT GAA Gln Thr Asn Lys Thr Leu Val Glu Ile Pro Glu Met Val Ala Thr Glu 1535 1540 1545	4782
GCT GCC TTT CTA TGC TTC ATG TAC AAT TTG CCA GTG ACA ACA CAG AGT Ala Ala Phe Leu Cys Phe Met Tyr Asn Leu Pro Val Thr Thr Gln Ser 1550 1555 1560	4830
GTT TCA ACC ACA CTG CTG GAA AAT GCC ACA TTA TTA CAA GCT AGA ACT Val Ser Thr Thr Leu Leu Glu Asn Ala Thr Leu Leu Gln Ala Arg Thr 1565 1570 1575	4878
ATG GCA CAG TTT GAG CTA TCA TAT TTT TAC ACA ATT AAT TTT GTG CGA Met Ala Gln Phe Glu Leu Ser Tyr Phe Tyr Thr Ile Asn Phe Val Arg 1580 1585 1590	4926
TTT GAT GGT AGT ATG CAT CCA GTC ATA CAT GAC AAG CTG AAG CGC TTT Phe Asp Gly Ser Met His Pro Val Ile His Asp Lys Leu Lys Arg Phe 1595 1600 1605 1610	4974
AAG CTA CAC ACT TGT GAG ACA TTC CTC AAT AAG TTG GCG ATC CCA AAT Lys Leu His Thr Cys Glu Thr Phe Leu Asn Lys Leu Ala Ile Pro Asn 1615 1620 1625	5022
AAA GGC TTA TCC TCT TGG CTT ACG AGT GGA GAG TAT AAG CGA CTT GGT Lys Gly Leu Ser Ser Trp Leu Thr Ser Gly Glu Tyr Lys Arg Leu Gly 1630 1635 1640	5070
TAC ATA GCA GAG GAT GCT GGC ATA AGA ATC CCA TTC GTG TGC AAA GAA Tyr Ile Ala Glu Asp Ala Gly Ile Arg Ile Pro Phe Val Cys Lys Glu 1645 1650 1655	5118
ATT CCA GAC TCC TTG CAT GAG GAA ATT TGG CAC ATT GTA GTC GCC CAT Ile Pro Asp Ser Leu His Glu Glu Ile Trp His Ile Val Val Ala His 1660 1665 1670	5166
AAA GGT GAC TCG GGT ATT GGG AGG CTC ACT AGC GTA CAG GCA GCA AAG Lys Gly Asp Ser Gly Ile Gly Arg Leu Thr Ser Val Gln Ala Ala Lys 1675 1680 1685 1690	5214
GTT GTT TAT ACT CTG CAA ACG GAT GTG CAC TCA ATT GCG AGG ACT CTA Val Val Tyr Thr Leu Gln Thr Asp Val His Ser Ile Ala Arg Thr Leu 1695 1700 1705	5262
GCA TGC ATC AAT AGA CGC ATA GCA GAT GAA CAA ATG AAG CAG AGT CAT Ala Cys Ile Asn Arg Arg Ile Ala Asp Glu Gln Met Lys Gln Ser His 1710 1715 1720	5310
TTT GAA GCC GCA ACT GGG AGA GCA TTT TCC TTC ACA AAT TAC TCA ATA Phe Glu Ala Ala Thr Gly Arg Ala Phe Ser Phe Thr Asn Tyr Ser Ile 1725 1730 1735	5358
CAA AGC ATA TTT GAC ACG CTG AAA GCA AAT TAT GCT ACA AAG CAT ACG Gln Ser Ile Phe Asp Thr Leu Lys Ala Asn Tyr Ala Thr Lys His Thr 1740 1745 1750	5406
AAA GAA AAT ATT GCA GTG CTT CAG CAG GCA AAA GAT CAA TTG CTA GAG Lys Glu Asn Ile Ala Val Leu Gln Gln Ala Lys Asp Gln Leu Leu Glu 1755 1760 1765 1770	5454

FIG. 1

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TTT TCG AAC CTA GCA AAG GAT CAA GAT GTC ACG GGT ATC ATC CAA GAC Phe Ser Asn Leu Ala Lys Asp Gln Asp Val Thr Gly Ile Ile Gln Asp 1775 1780 1785	5502
TTC AAT CAC CTG GAA ACT ATC TAT CTC CAA TCA GAT AGC GAA GTG GCT Phe Asn His Leu Glu Thr Ile Tyr Leu Gln Ser Asp Ser Glu Val Ala 1790 1795 1800	5550
AAG CAT CTG AAG CTT AAA AGT CAC TGG AAT AAA AGC CAA ATC ACT AGG Lys His Leu Lys Leu Lys Ser His Trp Asn Lys Ser Gln Ile Thr Arg 1805 1810 1815	5598
GAC ATC ATA ATA GCT TTG TCT GTG TTA ATT GGT GGT GGA TGG ATG CTT Asp Ile Ile Ile Ala Leu Ser Val Leu Ile Gly Gly Gly Trp Met Leu 1820 1825 1830	5646
GCA ACG TAC TTC AAG GAC AAG TTC AAT GAA CCA GTC TAT TTG CAA GGG Ala Thr Tyr Phe Lys Asp Lys Phe Asn Glu Pro Val Tyr Phe Gln Gly 1835 1840 1845 1850	5694
AAG AAG AAT CAG AAG CAC AAG CTT AAG ATG AGA GAG GCG CGT GGG GCT Lys Lys Asn Gln Lys His Lys Leu Lys Met Arg Glu Ala Arg Gly Ala 1855 1860 1865	5742
AGA GGG CAA TAT GAG GTT GCA GCG GAG CCA GAG GCG CTA GAA CAT TAC Arg Gly Gln Tyr Glu Val Ala Ala Glu Pro Glu Ala Leu Glu His Tyr 1870 1875 1880	5790
TTT GGA AGC GCA TAT AAT AAC AAA GGA AAG CGC AAG GGC ACC ACG AGA Phe Gly Ser Ala Tyr Asn Asn Lys Gly Lys Arg Lys Gly Thr Thr Arg 1885 1890 1895	5838
GGA ATG GGT GCA AAG TCT CGG AAA TTC ATA AAC ATG TAT GGG TTT GAT Gly Met Gly Ala Lys Ser Arg Lys Phe Ile Asn Met Tyr Gly Phe Asp 1900 1905 1910	5886
CCA ACT GAT TTT TCA TAC ATT AGG TTT GTG GAT CCA TTG ACA GGT CAC Pro Thr Asp Phe Ser Tyr Ile Arg Phe Val Asp Pro Leu Thr Gly His 1915 1920 1925 1930	5934
ACT ATT GAT GAG TCC ACA AAC GCA CCT ATT GAT TTA GTG CAG CAT GAG Thr Ile Asp Glu Ser Thr Asn Ala Pro Ile Asp Leu Val Gln His Glu 1935 1940 1945	5982
TTT GGA AAG GTT AGA ACA CGC ATG TTA ATT GAC GAT GAG ATA GAG CCT Phe Gly Lys Val Arg Thr Arg Met Leu Ile Asp Asp Glu Ile Glu Pro 1950 1955 1960	6030
CAA AGT CTT AGC ACC CAC ACC ACA ATC CAT GCT TAT TTG GTG AAT AGT Gln Ser Leu Ser Thr His Thr Ile His Ala Tyr Leu Val Asn Ser 1965 1970 1975	6078
GGC ACG AAG AAA GTT CTT AAG GTT GAT TTA ACA CCA CAC TCG TCG CTA Gly Thr Lys Lys Val Leu Lys Val Asp Leu Thr Pro His Ser Ser Leu 1980 1985 1990	6126
CGT GCG AGT GAG AAA TCA ACA GCA ATA ATG GGA TTT CCT GAA AGG GAG Arg Ala Ser Glu Lys Ser Thr Ala Ile Met Gly Phe Pro Glu Arg Glu 1995 2000 2005 2010	6174
AAT GAA TTG CGT CAA ACC GGC ATG GCA GTG CCA GTG GCT TAT GAT CAA Asn Glu Leu Arg Gln Thr Gly Met Ala Val Pro Val Ala Tyr Asp Gln 2015 2020 2025	6222

FIG. 1

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TTG CCA CCA AAG AAT GAG GAC TTG ACG TTT GAA GGA GAA AGC TTG TTT Leu Pro Pro Lys Asn Glu Asp Leu Thr Phe Glu Gly Glu Ser Leu Phe 2030 2035 2040	6270
AAG GGA CCA CGT GAT TAC AAC CCG ATA TCG AGC ACC ATT TGT CAT TTG Lys Gly Pro Arg Asp Tyr Asn Pro Ile Ser Ser Thr Ile Cys His Leu 2045 2050 2055	6318
ACG AAT GAA TCT GAT GGG CAC ACA ACA TCG TTG TAT GGT ATT GGA TTT Thr Asn Glu Ser Asp Gly His Thr Ser Leu Tyr Gly Ile Gly Phe 2060 2065 2070	6366
GGT CCC TTC ATC ATT ACA AAC AAG CAC TTG TTT AGA AGA AAT AAT GGA Gly Pro Phe Ile Ile Thr Asn Lys His Leu Phe Arg Arg Asn Asn Gly 2075 2080 2085 2090	6414
ACA CTG TTG GTC CAA TCA CTA CAT GGT GTA TTC AAG GTC AAG AAC ACC Thr Leu Leu Val Gln Ser Leu His Gly Val Phe Lys Val Lys Asn Thr 2095 2100 2105	6462
ACG ACT TTG CAA CAA CAC CTC ATT GAT GGG AGG GAC ATG ATA ATT ATT Thr Thr Leu Gln Gln His Leu Ile Asp Gly Arg Asp Met Ile Ile Ile 2110 2115 2120	6510
CGC ATG CCT AAG GAT TTC CCA CCA TTT CCT CAA AAG CTG AAA TTT AGA Arg Met Pro Lys Asp Phe Pro Pro Gln Lys Leu Lys Phe Arg 2125 2130 2135	6558
GAG CCA CAA AGG GAA GAG CGC ATA TGT CTT GTG ACA ACC AAC TTC CAA Glu Pro Gln Arg Glu Glu Arg Ile Cys Leu Val Thr Thr Asn Phe Gln 2140 2145 2150	6606
ACT AAG AGC ATG TCT AGC ATG GTG TCA GAC ACT AGT TGC ACA TTC CCT Thr Lys Ser Met Ser Met Val Ser Asp Thr Ser Cys Thr Phe Pro 2155 2160 2165 2170	6654
TCA TCT GAT GGC ATA TTC TGG AAG CAT TGG ATT CAA ACC AAG GAT GGG Ser Ser Asp Gly Ile Phe Trp Lys His Trp Ile Gln Thr Lys Asp Gly 2175 2180 2185	6702
CAG TGT GGC AGT CCA TTA GTA TCA ACT AGA GAT GGG TTC ATT GTT GGT Gln Cys Gly Ser Pro Leu Val Ser Thr Arg Asp Gly Phe Ile Val Gly 2190 2195 2200	6750
ATA CAC TCA GCA TCG AAT TTC ACC AAC ACA AAC AAT TAT TTC ACA AGC Ile His Ser Ala Ser Asn Phe Thr Asn Thr Asn Asn Tyr Phe Thr Ser 2205 2210 2215	6798
G TG CCG AAA AAC TTC ATG GAA TTG TTG ACA AAT CAG GAG GCG CAG CAG Val Pro Lys Asn Phe Met Glu Leu Leu Thr Asn Gln Glu Ala Gln Gln 2220 2225 2230	6846
TGG GTT AGT GGT TGG CGA TTA AAT GCT GAC TCA GTA TTG TGG GGG GGC Trp Val Ser Gly Trp Arg Leu Asn Ala Asp Ser Val Leu Trp Gly Gly 2235 2240 2245 2250	6894
CAT AAA GTT TTC ATG AGC AAA CCT GAA GAG CCT TTT CAG CCA GTT AAG His Lys Val Phe Met Ser Lys Pro Glu Glu Pro Phe Gln Pro Val Lys 2255 2260 2265	6942
GAA GCG ACT CAA CTC ATG AAT GAA TTG GTG TAC TCG CAA GGG GAG AAG Glu Ala Thr Gln Leu Met Asn Glu Leu Val Tyr Ser Gln Gly Glu Lys 2270 2275 2280	6990

FIG. 1

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AGG AAA TGG GTC GTG GAA GCA CTG TCA GGG AAC TTG AGG CCA GTG GCT Arg Lys Trp Val Val Glu Ala Leu Ser Gly Asn Leu Arg Pro Val Ala 2285 2290 2295	7038
GAG TGT CCC AGT CAG TTA GTC ACA AAG CAT GTG GTT AAA GGA AAG TGT Glu Cys Pro Ser Gln Leu Val Thr Lys His Val Val Lys Gly Lys Cys 2300 2305 2310	7086
CCC CTC TTT GAG CTC TAC TTG CAG TTG AAT CCA GAA AAG GAA GCA TAT Pro Leu Phe Glu Leu Tyr Leu Gln Leu Asn Pro Glu Lys Glu Ala Tyr 2315 2320 2325 2330	7134
TTT AAA CCG ATG ATG GGA GCA TAT AAG CCA AGT CGA CTT AAT AGA GAG Phe Lys Pro Met Met Gly Ala Tyr Lys Pro Ser Arg Leu Asn Arg Glu 2335 2340 2345	7182
GCG TTC CTC AAG GAC ATT CTA AAA TAT GCT AGT GAA ATT GAG ATT GGG Ala Phe Leu Lys Asp Ile Leu Lys Tyr Ala Ser Glu Ile Glu Ile Gly 2350 2355 2360	7230
AAT GTG GAT TGT GAC TTG CTG GAG CTT GCA ATA AGC ATG CTC GTC ACA Asn Val Asp Cys Asp Leu Leu Glu Leu Ala Ile Ser Met Leu Val Thr 2365 2370 2375	7278
AAG CTC AAG GCG TTA GGA TTC CCA ACT GTG AAC TAC ATC ACT GAC CCA Lys Leu Lys Ala Leu Gly Phe Pro Thr Val Asn Tyr Ile Thr Asp Pro 2380 2385 2390	7326
GAG GAA ATT TTT AGT GCA TTG AAT ATG AAA GCA GCT ATG GGA GCA CTA Glu Glu Ile Phe Ser Ala Leu Asn Met Lys Ala Ala Met Gly Ala Leu 2395 2400 2405 2410	7374
TAC AAA GGC AAG AAG AAA GAA GCT CTC AGC GAG CTC ACA CTA GAT GAG Tyr Lys Gly Lys Lys Glu Ala Leu Ser Glu Leu Thr Leu Asp Glu 2415 2420 2425	7422
CAG GAG GCA ATG CTC AAA GCA AGT TGC CTG CGA CTG TAT ACG GGA AAG Gln Glu Ala Met Leu Lys Ala Ser Cys Leu Arg Leu Tyr Thr Gly Lys 2430 2435 2440	7470
TTG GGA ATT TGG AAT GGC TCA TTG AAA GCA GAG TTG CGT CCA ATT GAG Leu Gly Ile Trp Asn Gly Ser Leu Lys Ala Glu Leu Arg Pro Ile Glu 2445 2450 2455	7518
AAG GTT GAA AAC AAC AAA ACG CGA ACT TTC ACA GCA GCA CCA ATA GAC Lys Val Glu Asn Asn Lys Thr Arg Thr Phe Thr Ala Ala Pro Ile Asp 2460 2465 2470	7566
ACT CTT CTT GCT GGT AAA GTT TGC GTG GAT GAT TTC AAC AAT CAA TTT Thr Leu Leu Ala Gly Lys Val Cys Val Asp Asp Phe Asn Asn Gln Phe 2475 2480 2485 2490	7614
TAT GAT CTC AAC ATA AAG GCA CCA TGG ACA GTT GGT ATG ACT AAG TTT Tyr Asp Leu Asn Ile Lys Ala Pro Trp Thr Val Gly Met Thr Lys Phe 2495 2500 2505	7662
TAT CAG GGG TGG AAT GAA TTG ATG GAG GCT TTA CCA AGT GGG TGG GTG Tyr Gln Gly Trp Asn Glu Leu Met Glu Ala Leu Pro Ser Gly Trp Val 2510 2515 2520	7710
TAT TGT GAC GCT GAT GGT TCG CAA TTC GAC AGT TCC TTG ACT CCA TTC Tyr Cys Asp Ala Asp Gly Ser Gln Phe Asp Ser Ser Leu Thr Pro Phe 2525 2530 2535	7758

FIG. 1

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CTC ATT AAT GCT GTA TTG AAA GTG CGA CTT GCC TTC ATG GAG GAA TGG Leu Ile Asn Ala Val Leu Lys Val Arg Leu Ala Phe Met Glu Glu Trp 2540 2545 2550	7806
GAT ATT GGT GAG CAA ATG CTG CGA AAT TTG TAC ACT GAG ATA GTG TAT Asp Ile Gly Glu Gln Met Leu Arg Asn Leu Tyr Thr Glu Ile Val Tyr 2555 2560 2565 2570	7854
ACA CCA ATC CTC ACA CCG GAT GGT ACT ATC ATT AAG AAG CAT AAA GGC Thr Pro Ile Leu Thr Pro Asp Gly Thr Ile Ile Lys Lys His Lys Gly 2575 2580 2585	7902
AAC AAT AGC GGG CAA CCT TCA ACA GTG GTG GAC AAC ACA CTC ATG GTC Asn Asn Ser Gly Gln Pro Ser Thr Val Val Asp Asn Thr Leu Met Val 2590 2595 2600	7950
ATT ATT GCA ATG TTA TAC ACA TGT GAG AAG TGT GGA ATC AAC AAG GAA Ile Ile Ala Met Leu Tyr Thr Cys Glu Lys Cys Gly Ile Asn Lys Glu 2605 2610 2615	7998
GAG ATT GTG TAT TAC GTC AAT GGC GAT GAC CTA TTG ATT GCC ATT CAC Glu Ile Val Tyr Tyr Val Asn Gly Asp Asp Leu Leu Ile Ala Ile His 2620 2625 2630	8046
CCA GAT AAA GCT GAG AGG TTG AGT AGA TTC AAA GAA TCT TTC GGA GAG Pro Asp Lys Ala Glu Arg Leu Ser Arg Phe Lys Glu Ser Phe Gly Glu 2635 2640 2645 2650	8094
TTG GGC CTG AAA TAT GAA TTT GAC TGT ACC ACC AGG GAC AAG ACA CAG Leu Gly Leu Lys Tyr Glu Phe Asp Cys Thr Thr Arg Asp Lys Thr Gln 2655 2660 2665	8142
TTG TGG TTC ATG TCA CAC AGG GCT TTG GAG AGG GAT GGC ATG TAT ATA Leu Trp Phe Met Ser His Arg Ala Leu Glu Arg Asp Gly Met Tyr Ile 2670 2675 2680	8190
CCA AAG CTA GAA GAA GAA AGG ATT GTT TCT ATT TTG GAA TGG GAC AGA Pro Lys Leu Glu Glu Arg Ile Val Ser Ile Leu Glu Trp Asp Arg 2685 2690 2695	8238
TCC AAA GAG CCG TCA CAT AGG CTT GAA GCC ATC TGT GCA TCA ATG ATT Ser Lys Glu Pro Ser His Arg Leu Glu Ala Ile Cys Ala Ser Met Ile 2700 2705 2710	8286
GAA GCA TGG GGT TAT GAC AAG CTG GTT GAA GAA ATC CGC AAT TTC TAT Glu Ala Trp Gly Tyr Asp Lys Leu Val Glu Glu Ile Arg Asn Phe Tyr 2715 2720 2725 2730	8334
GCA TGG GTT TTG GAA CAA GCG CCG TAT TCA CAG CTT GCA GAA GAA GGA Ala Trp Val Leu Glu Gln Ala Pro Tyr Ser Gln Leu Ala Glu Glu Gly 2735 2740 2745	8382
AAG GCG CCA TAT CTG GCT GAG ACT GCG CTT AAG TTT TTG TAC ACA TCT Lys Ala Pro Tyr Leu Ala Glu Thr Ala Leu Lys Phe Leu Tyr Thr Ser 2750 2755 2760	8430
CAG CAC GGA ACA AAC TCT GAG ATA GAA GAG TAT TTA AAA GTG TTG TAT Gln His Gly Thr Asn Ser Glu Ile Glu Glu Tyr Leu Lys Val Leu Tyr 2765 2770 2775	8478
GAT TAC GAT ATT CCA ACG ACT GAG AAT CTT TAT TTT CAG AGT GGC ACT Asp Tyr Asp Ile Pro Thr Thr Glu Asn Leu Tyr Phe Gln Ser Gly Thr 2780 2785 2790	8526

FIG. 1

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GTG GAT GCT GGT GCT GAC GCT GGT AAG AAG AAA GAT CAA AAG GAT GAT Val Asp Ala Gly Ala Asp Ala Gly Lys Lys Lys Asp Gln Lys Asp Asp 2795 2800 2805 2810	8574
AAA GTC GCT GAG CAG GCT TCA AAG GAT AGG GAT GTT AAT GCT GGA ACT Lys Val Ala Glu Gln Ala Ser Lys Asp Arg Asp Val Asn Ala Gly Thr 2815 2820 2825	8622
TCA GGA ACA TTC TCA GTT CCA CGA ATA AAT GCT ATG GCC ACA AAA CTT Ser Gly Thr Phe Ser Val Pro Arg Ile Asn Ala Met Ala Thr Lys Leu 2830 2835 2840	8670
CAA TAT CCA AGG ATG AGG GGA GAG GTG GTT GTA AAC TTG AAT CAC CTT Gln Tyr Pro Arg Met Arg Gly Glu Val Val Asn Leu Asn His Leu 2845 2850 2855	8718
TTA GGA TAC AAG CCA CAG CAA ATT GAT TTG TCA AAT GCT CGA GCC ACA Leu Gly Tyr Lys Pro Gln Gln Ile Asp Leu Ser Asn Ala Arg Ala Thr 2860 2865 2870	8766
CAT GAG CAG TTT GCC GCG TGG CAT CAG GCA GTG ATG ACA GCC TAT GGA His Glu Gln Phe Ala Ala Trp His Gln Ala Val Met Thr Ala Tyr Gly 2875 2880 2885 2890	8814
GTG AAT GAA GAG CAA ATG AAA ATA TTG CTA AAT GGA TTT ATG GTG TGG Val Asn Glu Glu Gln Met Lys Ile Leu Leu Asn Gly Phe Met Val Trp 2895 2900 2905	8862
TGC ATA GAA AAT GGG ACT TCC CCA AAT TTG AAC GGA ACT TGG GTT ATG Cys Ile Glu Asn Gly Thr Ser Pro Asn Leu Asn Gly Thr Trp Val Met 2910 2915 2920	8910
ATG GAT GGT GAG GAT CAA GTT TCA TAC CCG CTG AAA CCA ATG GTT GAA Met Asp Gly Glu Asp Gln Val Ser Tyr Pro Leu Lys Pro Met Val Glu 2925 2930 2935	8958
AAC GCG CAG CCA ACA CTG AGG CAA ATT ATG ACA CAC TTC AGT GAC CTG Asn Ala Gln Pro Thr Leu Arg Gln Ile Met Thr His Phe Ser Asp Leu 2940 2945 2950	9006
GCT GAA GCG TAT ATT GAG ATG AGG AAT AGG GAG CGA CCA TAC ATG CCT Ala Glu Ala Tyr Ile Glu Met Arg Asn Arg Glu Arg Pro Tyr Met Pro 2955 2960 2965 2970	9054
AGG TAT GGT CTA CAG AGA AAC ATT ACA GAC ATG AGT TTG TCA CGC TAT Arg Tyr Gly Leu Gln Arg Asn Ile Thr Asp Met Ser Leu Ser Arg Tyr 2975 2980 2985	9102
GCG TTC GAC TTC TAT GAG CTA ACT TCA AAA ACA CCT GTT AGA GCG AGG Ala Phe Asp Phe Tyr Glu Leu Thr Ser Lys Thr Pro Val Arg Ala Arg 2990 2995 3000	9150
GAG GCG CAT ATG CAA ATG AAA GCT GCT GCA GTA CGA AAC AGT GGA ACT Glu Ala His Met Gln Met Lys Ala Ala Val Arg Asn Ser Gly Thr 3005 3010 3015	9198
AGG TTA TTT GGT CTT GAT GGC AAC GTG GGT ACT GCA GAG GAA GAC ACT Arg Leu Phe Gly Leu Asp Gly Asn Val Gly Thr Ala Glu Glu Asp Thr 3020 3025 3030	9246
GAA CGG CAC ACA GCG CAC GAT GTG AAC CGT AAC ATG CAC ACA CTA TTA Glu Arg His Thr Ala His Asp Val Asn Arg Asn Met His Thr Leu Leu 3035 3040 3045 3050	9294

FIG. 1

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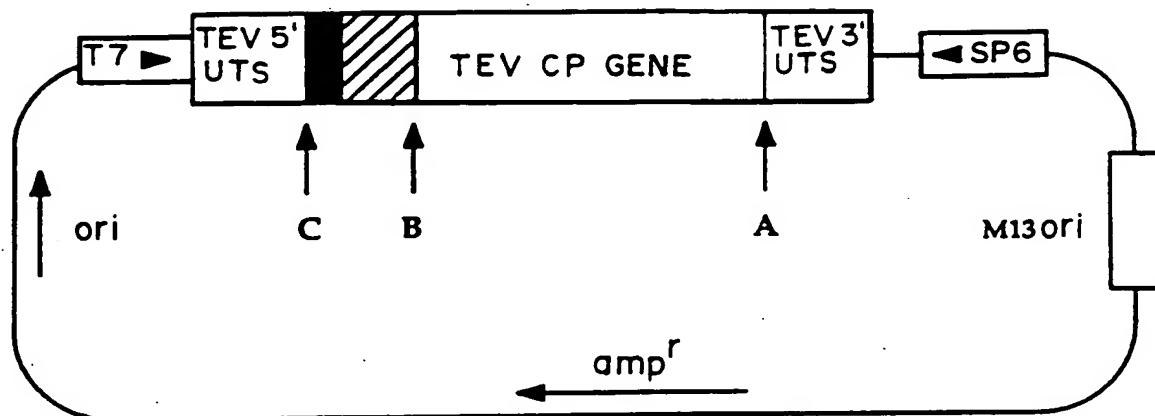
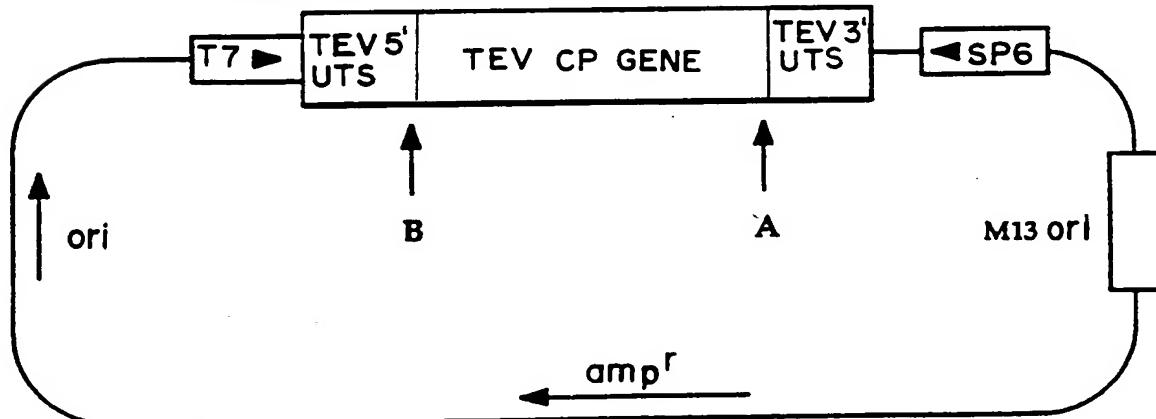
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Gly Val Arg Gln

GTAATATATA TGAATAGCTA TTCACAGTGG GACTTGGTCT TGTGTTGAAT AGTATCTTAT 9409

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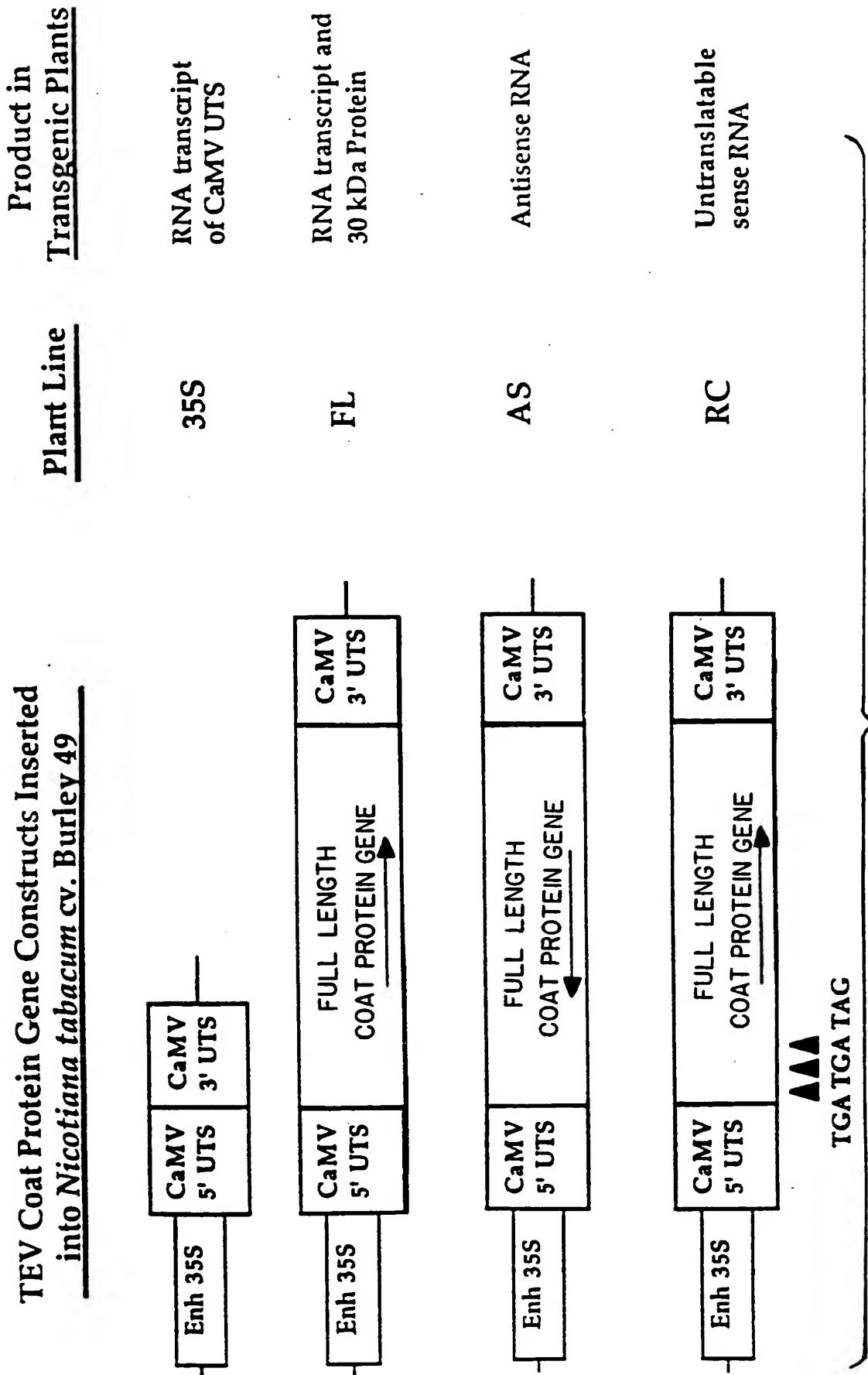
GGTCTAATTC TCCTATGTAG TGCGAG 9495

FIG. 1

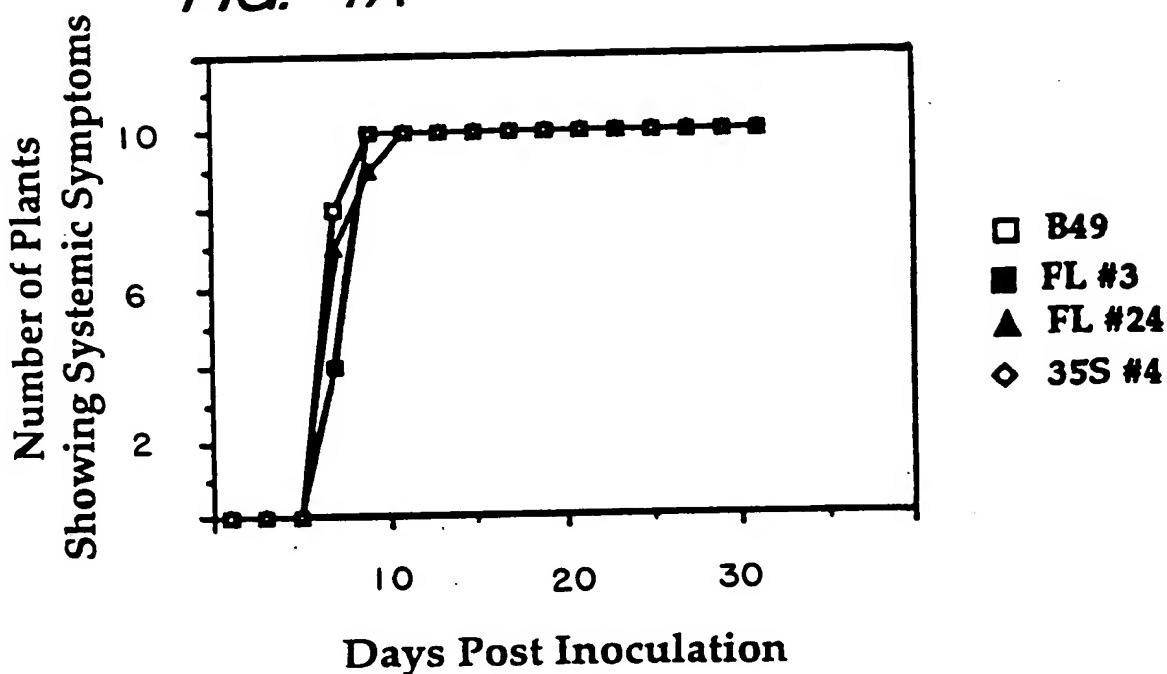
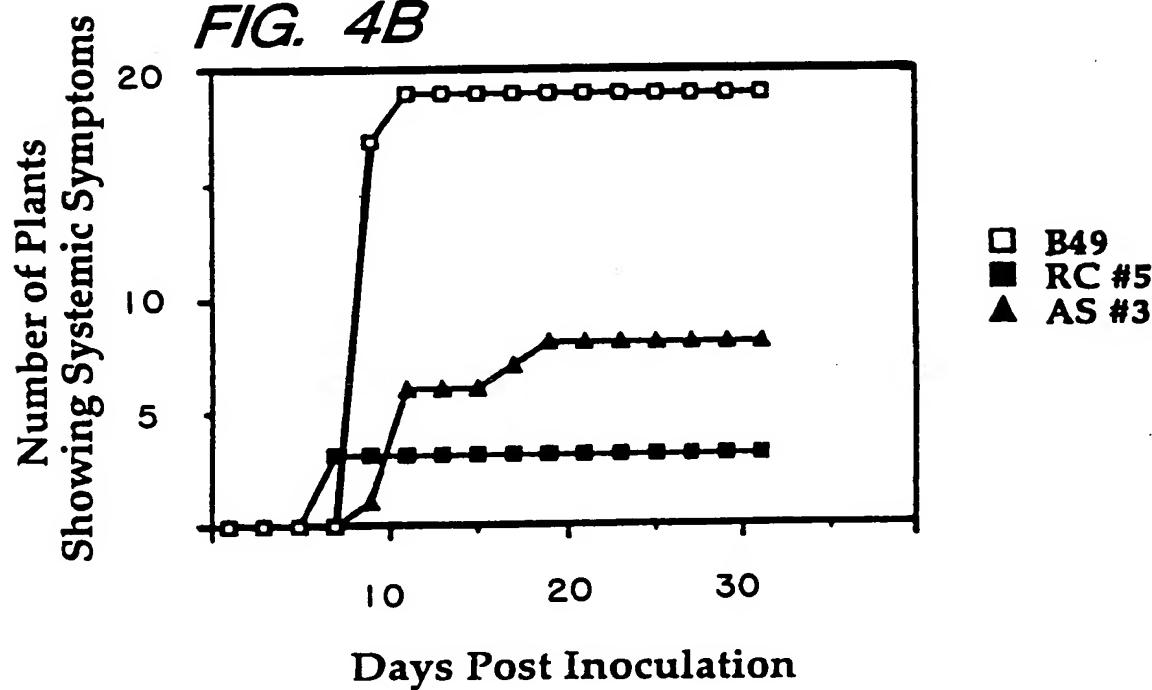
**pTL 37/8595**1. GENERATE BamHI SITE  
AT A(nt 9312-9317)2. GENERATE NcoI SITE  
AT B (nt 8516-8521)3. GENERATE BamHI SITE (nt 133-138)  
NcoI SITE (nt 143-148) AND  
DEOXYADENYLATE:  
RESIDUE (at nt 142) at C.**DIGEST WITH NcoI**REMOVE TEV NUCLEOTIDES 143-200/8462-8516  
(FLANKED BY SITES B AND C) AND RELIGATE.**pTC:FL****FIG. 2**

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TEV Coat Protein Gene Constructs Inserted  
into *Nicotiana tabacum* cv. Burley 49



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*FIG. 4A**FIG. 4B*

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US93/01544

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :C12N 1/21, 5/10, 15/33, 15/82; C07H 21/04; A01H 5/00

US CL :435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG,

search terms: virus or viral, untranslat?, resistan?

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Molecular Plant-Microbe Interactions, Volume 5, No. 2, issued March 1992, Lindbo et al, "Pathogen-derived resistance to a potyvirus: immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence", pages 144-153, see entire document.	1-27
X,P	Virology, Volume 189, No. 2, issued August 1992, Lindbo et al, "Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts", pages 725-733, see entire document.	1-27

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:		
*A* document defining the general state of the art which is not considered to be part of particular relevance	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*E* earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* document referring to an oral disclosure, use, exhibition or other means	*Z*	document member of the same patent family
*P* document published prior to the international filing date but later than the priority date claimed		

Date of actual completion of the international search

03 May 1993

Date of mailing of the international search report

26 MAY 1993

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Authorized officer

P. MOODY

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Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US93/01544**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :C12N 1/21, 5/10, 15/33, 15/82; C07H 21/04; A01H 5/00  
 US CL :435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/172.3, 240.4, 252.3, 320.1; 536/23.72; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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APS, DIALOG,  
 search terms: virus or viral, untranslat?, resistan?

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
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X,P	Virology, Volume 189, No. 2, issued August 1992, Lindbo et al, "Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts", pages 725-733, see entire document.	1-27

 Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of actual completion of the international search

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Washington, D.C. 20231

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